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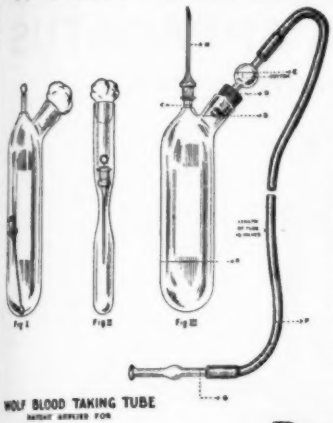

WOLF BLOOD TAKING TUBE

Instructions for Use

FIG. III.

"R." container capacity of 50 cubic centimeters; "B," tubulations; "C," ground nib; "D," perforated stopper; "E," trap containing cotton; "F," rubber tubing; "G" mouthpiece; "H," Luer needle.

For the purpose of elucidation in the use of the tube—To insure the positive exclusion of air it is advisable to smear a little vaseline around "C." Connect "D," "E," "F" and "G." Insert "D" in "B" and connect "H" and "C." Place the subject in a reclining position, flat on the back (this prevents to a great extent cerebral anemia) with the arm from which the blood is to be taken, in a suspended position. Select a prominent vein, sterilize the site, and apply the tourniquet. Place "G" in the mouth, produce suction, and insert the sterilized needle, taking the precaution to hold "B" upward (for if it is not in this position, the blood will gravitate into the tubulation and thereby be drawn into the mouth of the operator).

Description

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III. The tube can be cleansed and used over again.
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V. It is made to fit a 50 cc. centrifuge shield.
VI. Parts can be replaced independently.
VII. It is necessary to use but one hand, as the tube functions as a handle.
VIII. Blood is visible as soon as the needle pierces the wall of the blood vessel.
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All parts coming in contact with the tissue are of nickel plated brass, except the cutting discs which are of steel. The entire apparatus can be readily taken apart for cleaning and the clamp shown in the foreground is intended for convenient handling during sterilization.

For a recent reference to the use of this apparatus, see Alexis Carrel, M.D., "A Method for the Physiological Study of Tissues in Vitro," *The Journal of Experimental Medicine, Vol. XXXVIII, No. 4 (Oct. 1, 1923), p. 407.*

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THE DIGITALIS GLUCOSIDES.

I. DIGITOXIGENIN AND ISODIGITOXIGENIN.

By WALTER A. JACOBS and EDWIN L. GUSTUS.

(From the Laboratories of The Rockefeller Institute for Medical Research, New York.)

(Received for publication, May 3, 1928.)

Our investigations on the structure of strophanthidin were begun a number of years ago with the hope that the information obtained would be useful in similar studies with other members of the pharmacological group of substances to which it belongs. As previously presented,[1] the generally suspected close chemical relationship of many of these substances has been made more apparent by observations on their parallel behavior toward Tollens' reagent and sodium nitroprusside. These results have suggested that the aglucones of this group are $\Delta^{\beta,\gamma}$-lactones like strophanthidin in which the double bond may be found within or without the lactone ring. Attempts however to prove directly the presence of the $\Delta^{\beta,\gamma}$-lactone group in such substances as ouabain and the digitalis aglucones by the preparation of an oxime or a semicarbazone after gentle saponification of their lactone groups were without success.[2] A similar result was also obtained with certain strophanthidin derivatives. Fortunately, however, a means has now been found to accomplish this object, based upon the method recently used in the isostrophanthidin series.[3] In the present instance, we wish to present our experience with digitoxigenin.

In the case of isostrophanthidin, its lactone group has been shown to be the inner ester of the lactol form of a γ-hydroxyaldehyde, and that its formation from strophanthidin involves the

[1] Jacobs, W. A., and Hoffmann, A., *J. Biol. Chem.*, 1926, lxvii, 333.

[2] Jacobs, W. A., Hoffmann, A., and Gustus, E. L., *J. Biol. Chem.*, 1926, lxx, 1.

[3] Jacobs, W. A., and Gustus, E. L., *J. Biol. Chem.*, 1927, lxxiv, 811.

disappearance of the double bond of the latter with the production of a new ring. It was at first believed that this isomerization was precipitated by a shift in the double bond induced by enolization of the aldehyde group of strophanthidin under the influence of alkali. But it has now been found that a number of these aglucones which do not contain free carbonyl groups may be isomerized by alkali.

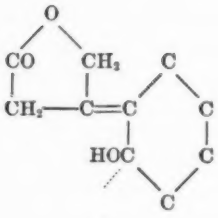
When digitoxigenin is dissolved in a methyl alcoholic solution of potassium hydroxide, the clear solution becomes, after a short time, a thick mass of lustrous, sparingly soluble crystals of a much higher melting point (271°) and proved to be of neutral character. Analysis showed the substance to possess, like digitoxigenin, the formula $C_{23}H_{34}O_4$. Contrary to digitoxigenin, *isodigitoxigenin* no longer gives the Legal test. On saponification it takes up 1 equivalent of alkali. When the solution of the salt is gently acidified with acetic acid a monobasic acid is obtained—*isodigitoxigeninic acid*. If an excess of mineral acid is employed, relactonization occurs with the reformation of isodigitoxigenin. Isodigitoxigeninic acid is identical with the so called dixgenic acid which was obtained by Kiliani[4] and Cloetta[5] over the sodium salt by the use of aqueous alcoholic alkali. Further study of isodigitoxigenin demonstrated that its relationship to digitoxigenin was similar to that already found between strophanthidin and its iso derivative, and that the transformations already reported in the case of the latter could be repeated exactly here. Contrary to digitoxigenin, isodigitoxigenin resembles isostrophanthidin and cannot be hydrogenated with palladium. The acid, isodigitoxigeninic acid, readily forms a methyl ester with diazomethane. This ester in turn was found to be the ester of a hydroxyaldehydo acid which may exist in either the free aldehydic or lactol form. On the one hand, the methyl ester readily formed a semicarbazone demonstrating the presence of the carbonyl group. On the other hand, if the ester in acetic acid solution was oxidized with chromic acid, it consumed exactly 2 atoms of oxygen with the formation of a neutral substance, $C_{24}H_{34}O_5$, which differed from the original material employed, $C_{24}H_{38}O_5$, by 4 hydrogen

[4] Kiliani, H., *Arch. Pharm.*, 1899, ccxxxvii, 452.
[5] Cloetta, M., *Arch. exp. Path. u. Pharmakol.*, 1920, lxxxviii, 138.

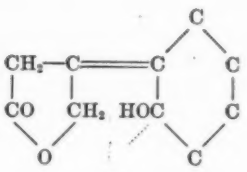
atoms. Although neutral in character, it was found to require 2 equivalents of alkali when saponified, due to the presence of a newly formed lactone group in addition to the original ester group. As seen from the analysis and its ability to form a semicarbazone the lactone ester contains also a carbonyl group so that during its formation the secondary alcoholic group of digitoxigenin is simultaneously oxidized to a ketone. The lactone group of this substance, therefore, could have been produced only by oxidation of the lactol form of a hydroxyaldehyde. After saponification, this lactone ester yielded on reacidification to Congo red a beautifully crystallizing acid which separated slowly as relactonization occurred. This acid, *isodigitoxigonic acid*, exhibited the proper behavior towards alkali. On direct titration it required 1 equivalent for neutralization, and on subsequent heating with an excess of reagent it consumed the additional equivalent required by the lactone group.

The analogy with isostrophanthidin was further confirmed by the behavior of isodigitoxigenin after saponification, that is as isodigitoxigeninic acid, towards hypobromite. This acid was oxidized presumably in the lactol form with the formation of the lactone acid, $C_{23}H_{34}O_5$, or *isodigitoxigenic acid* which differs from the isodigitoxigonic acid described above by the retention of the secondary hydroxyl group.

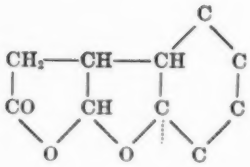
The results here obtained are exactly analogous to our experience with isostrophanthidin and give direct chemical evidence of the $\Delta^{\beta,\gamma}$ character of the lactone ring of digitoxigenin. Further, this lactone ring is attached by its β-carbon atom to a ring bearing a presumably tertiary hydroxyl group which in turn is most probably γ to the γ-carbon atom of the lactone ring. Alternative possibilities are given by placing the double bond $\Delta^{\beta,\gamma}$ either outside or inside of the lactone ring as shown in the partial formulæ (I) and (IV). In the case of formulæ (I) and (II) the opportunity for *cis-trans* isomerism with reference to the tertiary hydroxyl is shown and the possibility of this structural arrangement playing a rôle in the isomerization of strophanthidin, digitoxigenin, and other similar cardiac aglucones to iso compounds (III) will be discussed in another connection.



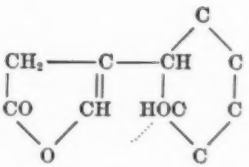
I.



II.



III.



IV.

Parallel studies have been made with gitoxigenin, but in this case a complication has been encountered which will be described in a communication soon to be published. The analytical data obtained with the digitoxigenin derivatives agree best with the figures required for a C_{23} formula, thus confirming the more recent conclusions of Windaus[6] that digitoxigenin is a C_{23} derivative and not a C_{24} compound as presented by Cloetta[7] and then provisionally accepted by Windaus and Freese.[8] Similarly, our analytical data obtained with the gitoxigenin and periplogenin derivatives have shown that these substances are likewise C_{23} derivatives.[9] Further work is in progress on the oxidative degradation of these substances as well as with attempts at their further correlation with strophanthidin. It is of particular interest to determine how far the structural analogies among these substances may be developed.

The digitoxigenin (digitoxin) employed in the present studies

[6] Windaus, A., *Nachr. Ges. Wissensch. Goettingen, Math.- physik. Klasse*, 1926, 170.

[7] Cloetta, M., *Arch. exp. Path. u. Pharmakol.*, 1920, lxxxviii, 133.

[8] Windaus, A., and Freese, C., *Ber. chem. Ges.*, 1925, lviii, 2503.

[9] In a private communication, Professor Windaus has stated that digitaligenin (dianhydrogitoxigenin) is also a C_{23} derivative.

was in part prepared by ourselves directly from digitalis leaves. But we are especially indebted to E. Merck and Company of Darmstadt to whose liberality we owe generous quantities of the insoluble by-product obtained in the manufacture of digitoxin and from which we have prepared both pure digitoxin and gitoxin.

EXPERIMENTAL.

Isodigitoxigenin.—0.2 gm. of digitoxigenin was dissolved in 2 cc. of a solution of potassium hydroxide in dry methyl alcohol (2.4 gm. per 100 cc.) and kept at 20°. After about 20 minutes, crystallization started and the mixture soon became a thick mass of glistening platelets. After 1 hour, the substance was collected and washed with 50 per cent alcohol. It proved to be neutral. The yield was 0.15 gm. Recrystallized from 95 per cent alcohol, it formed narrow platelets or needles which melted at 271°. It is very sparingly soluble in the cold, in methyl and ethyl alcohols, but is appreciably soluble in acetone and chloroform. It is practically insoluble in ether. Contrary to digitoxigenin, it gives no reaction with nitroprusside. In sulfuric acid it dissolves with a slowly developing fluorescent yellow color.

On acidification of the above alkaline mother liquor to Congo red, that portion of the substance which had undergone saponification was relactonized and proved to be identical with the isodigitoxigenin directly obtained.

In an attempt to hydrogenate isodigitoxigenin no hydrogen was absorbed. For analysis the substance was dried at 100° and 15 mm.

3.345 mg. substance: 2.800 mg. H_2O, 9.030 mg. CO_2.

$C_{23}H_{34}O_4$.	Calculated.	C 73.75, H 9.15.
	Found.	" 73.61, " 9.36.

Isodigitoxigeninic Acid (Dixgenic Acid).[4]—Isodigitoxigenin was gently heated in 50 per cent alcohol with a slight excess of dilute sodium hydroxide solution until it was dissolved. After cooling, gentle acidification with acetic acid caused the deposition of the acid as broad, flat micro spears, which were completely soluble in dilute carbonate solution. This substance melted with effer-

vescence on rapid heating at about 220°.[10] Upon recrystallization from 50 per cent alcohol, it separated as long, narrow platelets or broad, flat needles which were anhydrous and now melted at 250°.[10]

3.275 mg. substance: 2.785 mg. H_2O, 8.405 mg. CO_2.

$C_{23}H_{36}O_5$.	Calculated.	C 70.35, H 9.25.
	Found.	" 69.98, " 9.51.

Isodigitoxigeninic Methyl Ester.—On treatment of a suspension of the acid in dry acetone with diazomethane it slowly dissolved. The residue obtained after removal of the acetone crystallized under methyl alcohol. This was completed by careful dilution, broad six-sided plates being formed. From a small volume of methyl alcohol the ester slowly crystallized as beautifully formed prisms and tables about 0.5 cm. long, which melted at 128°. It is readily soluble in the usual solvents except ligroin. For analysis the ester was again recrystallized by dilution of its alcoholic solution.

3.530 mg. substance: 3.010 mg. H_2O, 9.165 mg. CO_2.

$C_{24}H_{38}O_5$.	Calculated.	C 70.88, H 9.43.
	Found.	" 70.80, " 9.54.

Isodigitoxigeninic Methyl Ester Semicarbazone.—0.1 gm. of the ester was dissolved in 10 cc. of alcohol and the solution was treated with 0.1 gm. of semicarbazide hydrochloride and 0.15 gm. of potassium acetate dissolved in 2 cc. of water. After standing 4 days at ordinary temperature, the clear solution was cautiously concentrated in a desiccator. The initial deposition of a gum was followed on rubbing by slow crystallization. Recrystallized by careful dilution of its methyl alcoholic solution, it gradually crystallized as stout prisms and tables which slowly effervesced at 156°. The substance which contained solvent of crystallization was dried for analysis at 100° and 15 mm.

4.260 mg. substance: 3.465 mg. H_2O, 10.030 mg. CO_2.

$C_{25}H_{41}O_5N_3$.	Calculated.	C 64.73, H 8.92.
	Found.	" 64.20, " 9.10.

Isodigitoxigonic Methyl Ester.—A solution of 0.7 gm. of isodigitoxigeninic methyl ester in 15 cc. of acetic acid was cooled and

[10] H. Kiliani gives for dixgenic acid the melting point, 220–230° (*Arch. Pharm.*, 1899, ccxxxvii, 452), and M. Cloetta, 243–244° (*Arch. exp. Path. u. Pharmakol.*, 1920, lxxxviii, 138).

treated with 3 cc. of Kiliani chromic acid solution (400 gm. of water, 80 gm. of H_2SO_4, and 53 gm. of CrO_3). Oxidation was prompt and after 5 minutes the mixture which contained a definite excess of the reagent was diluted with water. Glistening leaflets of the neutral lactone ester separated. The yield was 0.6 gm. Upon recrystallization from dilute methyl alcohol, it again separated as leaflets which melted at 190° after preliminary softening and were practically anhydrous. The ester is easily soluble in chloroform, acetone, and alcohol, and is appreciably soluble in ether. In sulfuric acid the solution is practically colorless.

4.660 mg. substance: 3.600 mg. H_2O, 12.175 mg. CO_2.
2.876 " " : 2.230 " " 7.533 " "
$C_{24}H_{34}O_6$. Calculated. C 71.60, H 8.52.
Found (*a*). " 71.24, " 8.64.
(*b*). " 71.42, " 8.67.

For the titration of the substance an as yet unpublished method devised by W. F. Goebel of the Rockefeller Institute was kindly placed at our disposal. For this purpose it was covered with 1 cc. of alcohol and refluxed in an atmosphere of washed N with 3 times the required 0.1 N NaOH solution for 4 hours and then titrated back with 0.1 N acid against phenolphthalein. 14.523 mg. of substance consumed 0.720 cc. of 0.1 N NaOH. Calculated for 2 equivalents, 0.722 cc. 0.1012 gm. of substance consumed 4.96 cc. Calculated for 2 equivalents, 5.03 cc.

Isodigitoxigonic Acid.—On acidification of the above solutions to Congo red, the lactone acid slowly crystallized as leaflets. Recrystallized by careful dilution of the alcoholic solution, it formed thin, anhydrous platelets which melted at 212–213° after slight preliminary sintering.

2.945 mg. substance: 2.195 mg. H_2O, 7.710 mg. CO_2.
$C_{22}H_{32}O_6$. Calculated. C 71.08, H 8.31.
Found. " 71.39, " 8.34.

11.622 mg. of substance were dissolved in 1 cc. of alcohol and directly titrated with 0.1 N NaOH. Found, 0.297 cc. Calculated for 1 equivalent, 0.299 cc.

The above solution was then treated with 1.4 cc. of 0.1 N NaOH and refluxed for 4 hours as described under the ester. Found, 0.295 cc. Calculated for 1 equivalent, 0.299 cc.

Isodigitoxigonic Methyl Ester Semicarbazone.—0.1 gm. of the ester dissolved in 10 cc. of methyl alcohol was allowed to stand with a solution of 0.1 gm. of semicarbazide hydrochloride and 0.15 gm. of potassium acetate at room temperature for several days. Careful dilution and evaporation gave at first a resin followed by slow crystallization. The collected substance was recrystallized by allowing the diluted methyl alcoholic solution to evaporate. The crystalline residue was collected with 70 per cent methyl alcohol.

The semicarbazone forms practically anhydrous microscopic aggregates of plates and rods which melted with effervescence at 243°.

For analysis, the substance was dried at 100° and 15 mm.

3.460 mg. substance: 2.616 mg. H_2O, 8.286 mg. CO_2.

$C_{25}H_{37}O_8N_3$.	Calculated.	C 65.31, H 8.12.
	Found.	" 65.30, " 8.44.

Isodigitoxigenic Acid.—0.15 gm. of isodigitoxigenin was dissolved in 2 cc. of pyridine and treated with 2 cc. of 0.5 N sodium hydroxide solution. The thick pap which formed cleared on gentle warming. After careful neutralization with acetic acid the cooled solution was treated with a solution of 0.15 gm. of bromine in 2.25 cc. of N sodium hydroxide solution. After standing 1 hour at ordinary temperature the reaction product was precipitated with hydrochloric acid and the amorphous acid was collected with water. By careful dilution of its solution in a small volume of alcohol it slowly crystallized as tables and prisms which were collected with cold 50 per cent alcohol. The substance which contained solvent of crystallization melted at 229° after preliminary softening and is readily soluble in alcohol, acetone, and chloroform and practically insoluble in water. In sulfuric acid it gradually forms a yellow solution which on long standing deepens to a deep green.

For analysis the substance was dried at 100° and 20 mm.

3.658 mg. substance: 2.926 mg. H_2O, 9.424 mg. CO_2.

$C_{23}H_{34}O_6$.	Calculated.	C 70.72, H 8.78.
	Found.	" 70.21, " 8.95.

11.315 mg. of the lactone acid were treated with 1 cc. of alcohol and directly titrated with 0.1 N NaOH against phenol-

phthalein. Found, 0.298 cc. Calculated for 1 equivalent, 0.291 cc.

The above solution was then treated with 3.5 cc. of 0.1 N NaOH and refluxed for 4 hours, and then titrated back. Found, 0.311 cc. Calculated for 1 equivalent, 0.291 cc.

HYDROGEN ION CONCENTRATION OF FISH MUSCLE.*

By C. C. BENSON.

(From the Lillian Massey Laboratory of Food Chemistry, University of Toronto, Toronto, and the Atlantic Biological Station, St. Andrews, New Brunswick, Canada.)

(Received for publication, May 3, 1928.)

In connection with studies of rigor mortis, it seemed worth while to try to find a satisfactory method of determining the hydrogen ion concentration of the muscle.

The quinhydrone method has proved very satisfactory for this purpose, for it allows rapid working and can be so arranged as to cause the minimum of injury to the tissue. Both of these conditions had to be met in these experiments, for Fletcher and Hopkins, (1), working with frog muscle have shown that injury to the tissue stimulates acid production, and Ritchie (2) and Simpson (3) have found for fish that muscle glycogen soon decreases after death, and lactic acid tends to increase very quickly.

Attempts were made to use muscle, frozen with solid carbon dioxide, but without success, and colorimetric determinations with extracts did not give values of sufficient exactness. Muscle, frozen in test-tubes in a brine bath, was used to make cold (3–4°) water extracts. Extracts were made also by dropping the excised muscle into ice water and filtering. The pH of these extracts was taken, quinhydrone being used, but these methods were all troublesome. A few of such determinations are here shown, for the sake of comparison, in Table I, which also shows that results obtained by the procedure described below gave similar results to those obtained with the more elaborate manipulations.

Most of the determinations were obtained by quickly cutting pieces of muscle from the fish immediately after it was stunned, covering a spot of the muscle with the powdered quinhydrone,

*The experimental work was done at the Atlantic Biological Station, St. Andrews, during 1927.

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and inserting a stout, bare platinum electrode in order to read the voltage against a calomel electrode with saturated potassium chloride connections. The readings were regularly made with two electrodes and gave the same values; sometimes the bridges,

TABLE I.

Comparison of pH Determined in Different Ways.

Fish No.	Relation to rigor.	pH				
		Quinhydrone method.			Indicator, brom-thymol blue.	
		On muscle.	With extract.	With juice.		
Determinations with trawl-caught haddock.						
6	Before.		6.67		6.4	Muscle frozen in brine bath, extracted with water.
	During.		6.67		6.5	
7	Before.		6.49		6.5	
	During.		6.49		6.5	
10	Before.	6.86	6.80			
14	Before.	6.70				Juice squeezed from muscle.
	During.	6.54		6.41		
	After.			6.50		
15	Before.			6.59		
	During.	6.53				
	After.			6.55		
9	Before.		6.28			Extract made by plunging muscle into ice water.
			6.38		6.4	
	During.	6.32	6.34			
	After.	6.43	6.47			
Determinations with minced beef.						
					H_2 electrode with extract.	
	After.	6.27	6.25		6.23	

(of 3 per cent agar and saturated potassium chloride), were changed, and frequently readings were duplicated by changing the places where the bridges made connection, or where the quinhydrone was placed.

In general, values duplicated well but sometimes variations were found as shown in Table II. These variations are to be expected for there are indications that during rigor the musculature of the fish does not change as a whole, but rather that changes are progressive and proceed more rapidly in one place than in another.

The chief difficulty experienced was in inserting the platinum electrode too deeply and so no doubt below the part of the tissue saturated with the quinhydrone.

The matter of temperature correction has also been troublesome for unfortunately the room temperatures during July, 1927, were often high at St. Andrews, where the measurements were made, and the fish muscle was consequently in many cases cooler than was the calomel electrode, for which no means of temperature adjustment had been provided. Biilmann and Krarup (4) have worked out a temperature correction factor for the quinhydrone electrode, and temperature corrections for the calomel electrode are given by Clark (5). Dawson (6) makes use of a formula combining these two factors,

$$\text{pH} = \frac{0.4561 - 0.00014t_1}{0.0541 + 0.002t_1} - \frac{v}{0.0541 + 0.002t} \text{ volts}$$

where v = the reading in volts, t = the temperature of the quinhydrone electrode, and t_1 = the temperature of the calomel electrode. This formula has been used in calculations from the voltage readings. I have also recorded in Table II the millivolts actually read as I have not yet been able to verify fully this correction factor for low temperatures with preparations such as these.

Trawl-Caught Fish.

Determinations were first made with haddock caught by trawl, and a typical experiment of this kind is given in Table II.

Other experiments of a similar kind gave values for pH as shown in Table III. At the bottom of this table are shown the times which the muscle took to reach maximum rigidity. These determinations were made at room temperature except in the case of Fish 12, which was kept in a cold chamber at 12° and was, therefore, slower in becoming hard. The values enclosed in parentheses are those near maximum rigidity.

TABLE II.

Determination of pH of Muscle of Trawl-Caught Haddock.

Fish 13; stunned when caught; maximum rigor (12°) in 5¾ hours.

Time after stunning.	Temperature.		Millivolts.	pH
	t	t_1		
min.	°C.	°C.		
3	16	21	53	6.85
5	16	21	62	6.69
9	17	21	59	6.75
15	18	21	64	6.66
30	21	21	64	6.69
36	22	22	72	6.51
hrs.				
2½	22	22	65	6.63
2½	25	25	60	6.72
3	25	25	56	6.74
4	25	25	67	6.52
5½	26	26	63,70	
24	22	22	72	6.51

TABLE III.

pH Values for Trawl-Caught Haddock.

Time after stunning.	Fish 6.	Fish 7.	Fish 11.	Fish 12.	Fish 14.	Fish 15.	Fish 20.
min.							
3	6.67	6.54	6.64	6.56			6.67
9			6.94	6.75			6.74
hrs.							
½			6.62	6.62	6.70	6.59	6.66
1			6.64	6.51			6.72
2+	(6.67)*	6.49	6.40		(6.54)	(6.53)	(6.68)
3+	6.54	(6.49)	6.40	6.44			6.57
4+			6.40	6.50	6.41		6.70
5½	6.49	6.49	6.42				6.66
20+	5.54			6.51	6.54	6.53	
Maximum rigor at.....	3−hrs.	3+hrs.		6 hrs.	3 hrs.	2+hrs.	3 hrs.

* The figures in parentheses denote values near maximum rigidity.

Penned Fish.

The muscle of fish, taken from a pen after 2 weeks or so of quiet, was found to have an alkaline reaction and to change as shown in Table IV.

From these measurements it is evident that the hydrogen ion concentration of muscle is very different for haddock taken from the pen (rested muscle) as compared with that from trawl-caught fish (fatigued muscle). As was to be expected, with penned fish, we find the muscle in alkaline condition; but with trawl-caught fish, the muscle is already near its condition of maximum acidity, due no doubt to the lactic acid produced by struggling.

TABLE IV.

Determinations of pH of Muscle of Haddock from Pen.

Time after stunning.	Fish 10.	Fish 16.	Fish 17.	Fish 21.	Fish 22.	Fish 23.
min.						
3	7.30	7.09	7.16	6.92	7.28	7.30
7	7.31	6.91	6.94	6.80	6.94	7.10
hrs.						
½	7.28	7.03	6.88	6.74	6.87	6.96
¾	7.26	6.98	6.88	6.80	6.74	6.93
1+	7.13	6.85	6.83	6.85	6.90	6.91
2+	6.88	6.75	6.74	6.74	6.81	7.00
3	6.86		6.94		6.71	
4	6.56	6.74	6.85			
7	6.54					
8	6.56					
9	6.48					
19		6.53				
Maximum rigor at.....	16½ hrs.*		16 hrs.*			

* Rigor measured at 12°.

Acidity and Rigor.

The maximum acidity seems, however, to be reached before the muscle showed marked evidence of rigor, and an examination of the variations of pH as compared with the increase and decrease of rigidity, indicates that, while the lower concentrations

of acid at the time of death are associated with the slower progress of rigor mortis, this condition of stiffening does not make itself evident with any definite change of pH value.

These determinations thus fit in with the conception of rigor mortis as a process indirectly related to the accumulation of lactic acid. When one compares the pH values with conditions of rigor mortis, at corresponding times, as found by Panton (7) and others, there is little obvious relationship except that noted by Leim, Macleod, and Simpson (8). This is the fact that the penned fish, which have much higher initial values for pH, pass into rigor much more slowly.

We thus see by actual measurement that the muscle of fish, when they are taken from the pen, is alkaline in reaction. The reaction changes soon to neutrality or to a faintly acid condition and then gradually grows more acid, probably to a pH of about 6.5 or possibly 6.4, though unfortunately it was not realized in time that it was wise to continue experiments long enough to see this, and the low value was only measured in two cases. With these fish, rigor was found to develop very slowly, but reached its maximum some time after the time of greatest acidity.

With trawl-caught fish, the muscle was never alkaline, when the fish were taken from the line. The amount of the acidity varied from a pH just at the neutral point to a value of between 6.5 and 6.6. The acidity increased, but not always regularly, and the minimum values of pH did not correspond with the onset of rigidity or its maximum. The accumulation of acid seems to be initiating some other change, which brings about the stiffening.

pH of Muscle of Hake, Cod, Eel-Pout, and Skate.

In Table V are also given results for a few determinations of pH with other species of fish, all caught by trawl.

These values agree in general with estimations of lactic acid made by Ritchie (9), who noted lower values of lactic acid for cod, than for haddock, and less change in reaction with change in condition of rigidity. He found still lower values for lactic acid in hake.

Further work should be done with skate muscle.

TABLE V.

Determination of pH of Muscle of Fish (Trawl-Caught).

Time after stunning.	Hake 2.	Hake 3.	Cod 1.	Cod 2.	Eel-pout.	Skate 1.	Skate 2.
min.							
6	7.06					7.12	
hrs.							
¼	7.33				6.53	6.82	
½	7.22			6.97		6.84	
¾	7.28						
1	7.20					6.54	6.56
2	7.24		6.96	6.76	6.54	6.65	6.50
3	7.16		6.90			6.29	
4+	7.10					6.34	
5+	7.22	7.17	7.08		6.44	6.40	
Maximum rigor at.....	1½ hrs.		2½ hrs.				

Degree of Acidity.

In regard to actual values for pH, these are of the same order of magnitude as those found for cat and for invertebrate muscle by Furusawa and Kerridge (10). Their measurements, taken immediately after death with quickly frozen muscle, varied between pH 6.98 to 7.13, with values, after 20 to 24 hours, of pH 6.10 to 6.52. The pH of 6.10, for tail muscle of *Scyllium canicula*, is lower than any of the values found for haddock muscle, where the lowest value for pH was usually about 6.40. In one case only, Haddock 9 (Table I), the value was as low as 6.28.

SUMMARY.

1. A method is described by which it has been possible to make consecutive measurements of hydrogen ion concentration of muscle over long periods at as frequent intervals as were needed.

2. Determinations of pH of haddock muscle show that fatigued muscle (trawl-caught) is acid at the time of death, may become a little more so, but varies only slightly during the changes of rigidity. The concentration of hydrogen ions is, therefore, not the cause of rigidity.

3. Determinations of pH with rested muscle of haddock (fish taken from a pen) showed an alkaline reaction. This soon changed

to the acid side and then gradually became more acid, approaching the values found for trawl-caught fish.

4. The pH of hake muscle (two tests) was alkaline during 5 hours after death, and never gave an acid reaction.

5. The pH of cod muscle (two tests) was near the neutral point and varied only slightly.

6. The muscle of skate grew slowly more acid in one experiment, of 4½ hours, while the muscle of an eel-pout, which had been out of water for ¾ hour and apparently dead for ¼ hour, showed an acid value for pH during 5 hours of testing.

BIBLIOGRAPHY.

1. Fletcher, W. M., and Hopkins, H. G., *J. Physiol.*, 1907, xxxv, 247.
2. Ritchie, A. D., *Brit. J. Exp. Biol.*, 1927, iv, 327.
3. Simpson, W. W., personal communication.
4. Biilmann, E., and Krarup, I., *J. Chem. Soc.*, 1924, cxxv, 1954.
5. Clark, W. M., The determination of hydrogen ions, Baltimore, 2nd edition, reprinted, 1927, 456.
6. Dawson, L. E., *Sugar*, 1926, xxviii, 212.
7. Panton, J. R., personal communication.
8. Leim, A. H., Macleod, J. J. R., and Simpson, W. W., *Contributions Canad. Biol. and Fisheries*, 1927, iii, 459.
9. Ritchie, A. D., *J. Physiol.*, 1926, lxi, p. iv.
10. Furusawa, K., and Kerridge, P. M. T., *J. Physiol.*, 1927, lxiii, 33; *J. Marine Biol. Assn. U. K.*, 1927, xiv, 657.

THE SOLUBILITY IN THE STOMACH AND DUODENUM OF ALUMINUM COMPOUNDS FOUND IN BAKING POWDER RESIDUES.

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The question has repeatedly been raised as to whether aluminum, when ingested with the food in an insoluble or largely insoluble form such as found in baking powder residues, goes into solution in the stomach. At the time this study was undertaken (1924) the evidence on this point was very limited, although a few *in vitro* and *in vivo* experiments had been conducted. The literature has recently been reviewed by Smith (1).

Experiments were accordingly carried out on human subjects to answer this question. Hospital patients, suffering from disorders which would not influence the results of this study, were given test breakfasts consisting of approximately 100 gm. of biscuit raised with several aluminum baking powders, together with 400 cc. of water. The biscuits were baked for us in the hospital diet kitchen. At the end of 1 hour the gastric contents were siphoned off and analyzed.

The gastric contents were filtered through heavy, folded filter paper, the fluid being returned to the filter if not water-clear. The acidities were titrated with the Töpfer method, while the pH was estimated with the aid of the Myers bicolorimeter, thymol blue being used as the indicator. Aluminum was determined by the Schmidt-Hoagland (2) method, the precipitates being washed with 1 per cent ammonium phosphate as suggested by Balls (3). This method was carefully checked and seemed well suited to the problem at hand. As an additional check on the composition of the aluminum phosphate precipitate, the phosphorus in the precipitate was determined by the Benedict and Theis (4) colori-

metric method and from this the amount of aluminum calculated. Excepting two experiments, the agreement was very close.

TABLE I.

Gastric Analyses on Subjects Receiving Baking Powder Biscuits.

Subject No.	Biscuits ingested.	Baking powder in biscuits ingested.	Gastric contents withdrawn.	pH	Free HCl.	Total acidity.
	gm.	*gm.*	*cc.*		*per cent*	*per cent*
1	100*	0	110	1.3	34	62
2	100	0	88		25	52
3	92	6.07	180	2.2	16	72
4	89	5.87	250	2.4	6	41
5	72	1.15	195	1.9	16	54
6	63	1.00	350	1.4	30	54
7	102	1.63	284	1.4	28	53
8	95	1.52	180	2.4	7	42
9	104	2.84	280	2.2	22	70
10	101	2.75	140	3.0	0	50
11	68	1.85	87	2.1	16	42
12	93	2.53	190	3.0	0	34
13	88	1.24	200			
14	83	1.17	345		0	54
15	89	1.26	214			
16	102	1.44	280			
17	94	1.33	200		18	39
18	108	1.94	175		21	50
19	94	1.78	210		26	65
20	64	1.12	200			

* Subjects 1 and 2 served as controls and received "soda" crackers and yeast bread respectively, Subjects 3 to 8 received biscuits baked with a combination acid calcium phosphate-sodium aluminum sulfate baking powder, while the biscuits given to Subjects 9 to 20 were raised with the aid of two different brands of straight sodium aluminum sulfate baking powder. The biscuits given to Subjects 3 and 4 contained 4 times the amount of baking powder required.

DISCUSSION.

It is believed that the gastric analyses recorded in Table I are fairly representative of what might be found in hospital patients after an Ewald meal containing the amount of bread and water administered. It will be noted in Table II that the gastric contents

removed contained in soluble form from 2 to 20 per cent of the aluminum administered, if the analysis of the filtered gastric contents may be considered as giving the soluble aluminum. In six of the subjects the total aluminum present in the gastric contents removed was also determined. This permitted the calculation of the percentage of soluble (filtrable) aluminum in the

TABLE II.

Soluble Aluminum in Gastric Contents from Baking Powder Residues.

Subject No.	Al in baking powder used.	Al in biscuits ingested.	Total Al in gastric contents.	Soluble Al in gastric contents.	Soluble Al calculated from the P of $AlPO_4$.	Soluble Al in per cent of total Al of gastric contents.	Soluble Al in per cent of Al ingested.
	per cent	*mg.*	*mg.*	*mg.*	*mg.*		
1	0	0		0.12			
2	0	0		0.07			
3	1.52	92.0		14.6	14.5		15.9
4	1.52	89.0		8.1			9.1
5	1.52	17.3		3.3	3.3		19.1
6	1.52	15.1		0.4	0.4		2.7
7	1.52	24.6		2.2	2.3		8.9
8	1.52	22.8		2.1	1.3		9.2
9	2.23	63.4		12.5	10.3		19.7
10	2.23	61.4		1.0	0.8		1.6
11	2.23	41.3		1.7			4.1
12	2.23	56.6		1.5	1.7		2.7
13	3.58	44.7	14.1	1.4		9.9	3.1
14	3.58	42.1	27.7	9.1		32.9	21.6
15	3.58	45.2	23.8	4.7		19.8	10.4
16	3.58	51.8	21.9	1.4		6.4	2.7
17	3.58	47.7	23.2	8.6		37.1	18.0
18	3.58	69.0		3.2			4.6
19	3.58	63.9	20.7	11.1		53.6	17.4
20	3.58	40.3		2.8			7.0

gastric contents. In the six subjects it varied from 6 to 54 per cent and averaged 27 per cent. As will be noted in the tables, there does not appear to be any direct relationship between the gastric acidity and the amount of the soluble aluminum.

If absorption of aluminum takes place, it probably does not occur in the stomach. For this reason observations were made on the content of aluminum in the duodenal contents of four subjects

after they had received 63 mg. of aluminum, on the average, in the form of baking powder biscuits. The duodenal contents were withdrawn with the aid of a duodenal tube inserted from 85 to 100 cm. The soluble aluminum in the four cases was 0.13, 0.55, 0.80, and 0.82, calculated as mg. of Al per 100 cc. In the last two cases the total Al per 100 cc. was 7.47 and 2.74 mg., indicating solubilities of 10.7 and 29.9 per cent respectively. Although our observations on duodenal contents are quite limited, when considered in connection with similar data on the gastric contents, it would seem that they admitted of but one interpretation; namely, that on the average about 25 per cent of the aluminum of baking powder residues found in the stomach and duodenum are present in soluble form. Despite this fact the absorption of aluminum appears to be extremely slight (5, 6).

SUMMARY.

The solubility in the stomach of aluminum compounds found in baking powder residues has been studied in eighteen subjects. With an ingestion of 15 to 90 mg. of aluminum from 0.4 to 14.6 mg. have been found in the gastric contents in soluble form, comprising from 2 to 20 per cent of the amount ingested. In six of the subjects the total aluminum of the gastric contents was also determined. The soluble aluminum, calculated on the basis of the amount actually present in the gastric contents, varied from 6 to 54 per cent, averaging about 25 per cent. The solubility of the aluminum did not seem to bear any direct relation to the gastric acidity. Somewhat similar observations were made on the solubility of aluminum in the duodenal contents of four subjects.

BIBLIOGRAPHY.

1. Smith, E. E., Aluminum compounds in food, New York, 1928, 189.
2. Schmidt, C. L. A., and Hoagland, D. R., *J. Biol. Chem.*, 1912, xi, 387.
3. Balls, A. K., The occurrence of aluminum, and its absorption from food, in dogs, unpublished dissertation, Columbia University, New York, 1917.
4. Benedict, S. R., and Theis, R. C., *J. Biol. Chem.*, 1924, lxi, 63.
5. Myers, V. C., and Mull, J. W., *J. Biol. Chem.*, 1928, lxxviii, 605.
6. Myers, V. C., and Morrison, D. B., *J. Biol. Chem.*, 1928, lxxviii, 615.

THE ESTIMATION OF ALUMINUM IN ANIMAL TISSUES.*

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The question of the absorption of aluminum from the alimentary tract and its presence in various tissues, when diets both with and without aluminum were fed, has been a topic of discussion for many years. No definite attempts were made to answer these questions, however, until about 1911 when several studies were reported from Gies' laboratory. Steel (1), Kahn (2), and later Balls (3) attempted to ascertain the presence of aluminum in normal tissues and studied the influence of ingested aluminum. Both Steel and Kahn employed the method of the Assqciation of Official Agricultural Chemists, slightly modified (4), while Balls used the later Schmidt and Hoagland (5) method. This method was also employed by the Referee Board (6) to estimate the aluminum content of the blood of subjects receiving aluminum from baking powder residues. The Schmidt-Hoagland method, in particular, is a most excellent one when the quantities of aluminum are sufficient for accurate gravimetric work, as for

* The data presented in this and the two following papers are taken from the dissertations submitted by James W. Mull and Dempsie B. Morrison to the Graduate College of the State University of Iowa, July, 1927, and June, 1928, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

A report of the work given in this and the three following papers was presented before the American Society of Biological Chemists, at Ann Arbor, Michigan, April 12, 1928. A preliminary report of the method was presented before the Iowa Branch of the Society for Experimental Biology and Medicine, February 2, 1927 (Mull, J. W., Morrison, D. B., and Myers, V. C., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 476).

example in the feces of aluminum-fed subjects (7), but the amounts of aluminum present in animal tissues are so small, and the inorganic constituents so large, that the data which have been reported with this and other gravimetric methods are of questionable value.

Since our work was completed a book by Smith (8) on aluminum compounds in foods has appeared, in which figures for a number of unpublished analyses are given. Some of these harmonize with our findings very well, notably those of Killian (9) and Gray (10). The former employed the Schmidt-Hoagland method, while the latter used the Atack (11) alizarin method, slightly modified.

In the present study a delicate colorimetric method has been used, which, although less accurate than gravimetric methods where large amounts are to be determined, is unquestionably better suited to the estimation of minute amounts of aluminum in animal tissues. Three very delicate color reagents for aluminum have been described: alizarin by Atack (11) in 1915, the ammonium salt of aurin tricarboxylic acid by Hammett and Sottery (12) in 1925, and morin by Schantl (13) in 1924, the last named having thus far been used only in a qualitative way. The method of Hammett and Sottery, with certain modifications, has proved most suitable, the aurin being less affected by slight changes in the acid concentration and showing less tendency to salt out than the alizarin used by Atack.

The amount of aluminum present in biological tissues is so small, in comparison with the other inorganic constituents, especially iron, that it was found necessary to remove these before making the colorimetric tests. The ordinary methods of separation proved quite inadequate for this as the small amounts of iron left are sufficient to give a color with the dye so similar to that of aluminum as to invalidate the results. It was later found that, although iron must be completely removed before the development of the color reaction, it must be present at the time of the first precipitation of the aluminum, otherwise the aluminum is not completely precipitated. The following method of separation and subsequent estimation has been worked out and found to be entirely successful for the purpose.

Method.

Digestion of Tissues.—The tissues to be analyzed are digested until all organic matter has been destroyed in Pyrex or silica flasks of a capacity convenient for the amount of tissue employed. 50 gm. of tissue may be handled in 300 cc. flasks, but this amount requires careful manipulation. Blanks run with both Pyrex and silica flasks have shown that either are suitable for the work. The digestions are made with a mixture of concentrated sulfuric acid and 60 per cent perchloric acid, roughly in the proportion of 1 to 1.25, with an amount that will keep the material semiliquid at all times. A few silica chips are an aid in overcoming the bumping which is apt to occur as the water vapor is driven off, just before charring, but which is usually not severe. The flasks are heated with a very low flame, so that the contents barely boil, the flame being removed if the action becomes intense. This method prevents a rather violent exothermic reaction which otherwise takes place, accompanied by a tremendous evolution of fumes. During the course of the digestion the material chars, becomes thick, then begins to clear up, the final product being a water-clear liquid, which generally contains a fine white deposit. This liquid should be concentrated to a small volume, 5 cc. or less. A suction tube, introduced part way down the neck of the flask, markedly accelerates this operation by removing the heavy acid fumes which are otherwise driven off with difficulty.

Removal of Iron and Salts.—The digested product, including the precipitate which is loosened with a glass rod and partially dissolved in concentrated HCl, is transferred to a volumetric flask and made up to volume. If a very little aluminum is expected the whole of the digestion is used, otherwise an aliquot is taken. The amount used is pipetted into a 50 cc. conical centrifuge tube and 1 mg. of ferric iron added, if the amount of iron in the tissue is very small. The contents are then diluted to about 15 cc. with distilled water, neutralized with ammonium hydroxide, 1 drop of very dilute methyl red being used as indicator, and 1 cc. of a saturated solution of ammonium acetate added.

The tubes are then placed in a boiling water bath for 10 minutes or more, until the precipitate has begun to settle, centrifuged, and the liquid decanted off and discarded. This removes all the

soluble salts. The precipitate is dissolved as completely as possible in 1 cc. of 6 N HCl, with warming when necessary, and the solution diluted to 15 cc. To this solution, cooled to room temperature, is added 1.25 cc. of glacial acetic acid and 5 cc. of 6 N NaOH prepared from metallic sodium (special aluminum-free). With occasional agitation the tubes are allowed to stand an hour, to insure complete precipitation of the iron and separation from aluminum, then, after washing down the sides with distilled H_2O, they are centrifuged, and the liquid decanted into 50 cc. Nessler tubes.

Development of the Color.—To the solution in the Nessler tubes are added 1.1 cc. of 6 N HCl and 0.75 cc. of glacial acetic acid and the volume made up to between 35 and 40 cc. with distilled water. A 0.1 per cent solution of the ammonium salt of aurin tricarboxylic acid[1] is then added in the proportion of 1 cc. for each 0.01 mg. of aluminum, the tubes inverted, and thoroughly shaken. 10 minutes are permitted for the formation of the lake in this acid solution, after which it is neutralized with an ammonium hydroxide-carbonate mixture. This is made by dissolving 180 gm. of $(NH_4)_2CO_3$ in 420 cc. of concentrated NH_4OH and 1700 cc. of water. 5 cc. of this mixture should bring the pH of the determination to between 7.0 and 7.3, but the amount may be varied if necessary. After the mixture is inverted and shaken thoroughly, the colors are compared against standards, the full length of the Nessler tubes being used. It is advisable to shake and invert all the tubes a fixed number of times in order that the evolution of CO_2 may be as nearly uniform as possible. The bubbles formed by the escape of this gas make the use of a colorimeter rather unsatisfactory. At first we employed a 100 mm. Duboscq colorimeter but later, owing to the bubbles and lightness of the colors, we abandoned this in favor of the 200 mm. Nessler tubes.

Fresh standards[2] are prepared for each determination. These

[1] Prepared by dissolving 1 gm. of aurin tricarboxylic acid (Eastman) in about 200 cc. of water and 2.5 cc. of concentrated NH_4OH and making up to 1 liter. The solution should not be left exposed to the light.

[2] A stock aluminum standard may be prepared by dissolving 3.5018 gm. of potassium alum in distilled water and diluting to 1 liter. 50 cc. of the stock solution make a liter of a standard, containing 0.01 mg. of Al in 1 cc. Our potassium alum was five times recrystallized and carefully dried over $CaCl_2$ under reduced pressure.

differ by 0.005 mg. of aluminum and cover the desired range, usually from 0.005 mg. to 0.035 mg., although from 0.00 to 0.06 mg. may be used. They are made by taking the necessary amounts of the standard aluminum solution, prepared from recrystallized potassium aluminum sulfate, and adding the same reagents used in the unknowns; *i.e.*, 5 cc. of NaOH, 2.1 cc. of 6 N HCl, 2 cc. of glacial acetic acid, the correct amount of dye, and ammonium hydroxide-carbonate mixture. The comparisons are made as soon as the bubbles pass off. The pH of the solutions is then taken colorimetrically, phenol red being used, and all tubes falling outside the range of 7.0 to 7.4 are discarded.

DISCUSSION.

It is of the greatest importance in using the method described to have pure reagents. The digesting acids must be free from aluminum and all materials used in the later steps must be free from iron as well. Satisfactory sodium hydroxide is particularly difficult to obtain. Only that prepared from metallic sodium can be used, and some brands of this contain enough iron or aluminum to make them unsuitable. The aurin tricarboxylic acid must be carefully tested, as some of the preparations on the market do not give good color lakes. Ammonium hydroxide which has stood for some time in glass may give a test for aluminum and in this case should be redistilled before being used.

The amount of aluminum present in the digestion as used is usually so small that it cannot, of itself, be precipitated by ammonium hydroxide. In the presence of iron, however, the precipitation is quantitative. It is therefore necessary to add iron to the aliquots taken, unless it is already present, even though it must be removed later to prevent interference with the aurin aluminum color lake. The effectiveness of the precipitation is also increased by the presence of the acetate ion with both the NH_4OH and NaOH. In the latter case satisfactory removal of the iron is entirely dependent on the amount of acetic acid used. Increasing the proportion causes loss of aluminum, while a decrease permits iron to go through.

In applying this method a number of limitations must be taken into consideration. First it is designed for very small amounts of aluminum. Determinations are not accurately made with

amounts of 0.1 mg. of aluminum or more and we personally find the range below 0.06 mg. preferable. We do not, therefore, recommend this method when the aluminum content exceeds 1.0 mg. per 100 gm. of tissue.

The nature and amount of the other inorganic constituents are also limiting factors. If the precipitate resulting from the neutralization with ammonium hydroxide is too great to be dissolved in 1 cc. of 6 N hydrochloric acid, even after dilution to 15 cc., the determination is impossible with the directions given. Similarly, if the iron precipitated by the sodium hydroxide forms a bulk of over 3 cc., after it has been centrifuged, it will hold aluminum and produce inaccurate results.

The most probable source of error in the method is apparently that of contaminations introduced during the digestion. We have checked this in several ways. The acids have been tested separately and have been found aluminum-free. Blanks have been run in which no tissue was used, to which not only iron but, at times, magnesium and calcium phosphate were added. These tests seem to check, and show a total of not more than 0.01 mg. of aluminum present in the entire digestion mixture. Since this amount represents a contamination present in almost all material, we have considered the digestion method satisfactory. The failure to find very little, if any, aluminum in certain tissues, notably blood, seems to strengthen our position.

The possible introduction of aluminum with the reagents subsequently used is of no consequence, since the same amounts of these reagents are also used in the standards. There remains the possibility of loss of aluminum, or of the presence of some other element that will form the color lake. The former possibility has been checked by adding aluminum to samples and carrying them through the process; the latter by making an analysis, adding aluminum to another aliquot, and finding the calculated total on the basis of the first analysis. It is improbable that any other element in the tissues would escape elimination and give a color addable to that of aluminum. Additional evidence is supplied by spectrograms of the digested material (kindly furnished by Dr. O. S. Rask) which show the lines characteristic of aluminum.

In Table I are presented experiments which show that iron, calcium, magnesium, and phosphate are removed with sufficient

TABLE I.

Checks on Purity of Reagents and Removal of Other Elements.

Materials added.							Al found.
85 per cent H_3PO_4.	Fe	Concentrated H_2SO_4.	Mg	$Ca_3(PO_4)_2$	$CaCl_2$	Al	
cc.	*mg.*	*cc.*	*mg.*			*mg.*	*mg.*
0.25	0.3	1				0.01	0.01
0.25	0.5	1				0.01	0.01
0.25	0.6	1				0.01	0.01
0.25	1.0	1				0.01	0.01
	1.0	1					0.001
	1.0	1				0.005	0.005
Present.	0.5				Present.		0.000
	0.3					0.01	0.01
	0.3				Present.	0.02	0.02
Present.	0.3				"	0.02	0.02
	1.0	1		Present.		0.015	0.015
	1.0	1	10			0.005	0.005

TABLE II.

Checks on Digestion and Recovery of Added Aluminum.

Materials present.					Al found.
H_2SO_4	$HClO_4$	Tissue.		Al	
cc.	*cc.*	*gm.*		*mg.*	*mg.*
15	20	*			0.01
15	20	†			0.01
15	20	Liver.	50.5		0.03
15	20	"	52	0.05	0.092
20	25	Kidney.	49.5		0.023
20	25	"	47.0	0.05	0.073
20	25	Heart.	50.8		0.120
20	25	"	51.2	0.05	0.160
20	25	Liver.	40.0		0.080
20	25	"	40.0	0.10	0.176
20	25	Heart.	40.0		0.036
20	25	"	40.0	0.07	0.100
20	25	Liver.	40.0		0.066
20	25	"	40.0	0.07	0.130
3	5	Blood.	5.0		0.005
3	5	"	5.0	0.01	0.013
3	5	"	5.0	0.02	0.024
3	5	"	5.0	0.03	0.033

*5 mg. of Fe added.

†5 mg. of Fe, 10 mg. of Mg, and 25 mg. of $Ca_3(PO_4)_2$ added.

TABLE III.

Checks on Final Colorimetric Readings.

Aliquot of tissue digestion.	Al added.	Al found.	Al in 5 cc. of digestion mixture calculated from determinations.
cc.	*mg.*	*mg.*	*mg.*
10	0.01	0.012	0.001
15		0.007	0.002
5		0.01	0.1
5	0.01	0.022	0.012
5	0.01	0.022	0.012
10		0.005	0.0025
15		0.007	0.0023
10	0.005	0.009	0.002
10	0.005	0.011	0.003
10		0.008	0.004
10		0.007	0.0035
5	0.01	0.012	0.002
5		0.002	0.002
5	0.005	0.007	0.002
10		0.007	0.0035
10		0.007	0.0035
15		0.008	0.0026
5	0.01	0.012	0.002
5	0.005	0.014	0.009
10		0.019	0.0095
5		0.013	0.013
5	0.005	0.018	0.013
4		0.011	0.013
5		0.012	0.012
5		0.012	0.012
5	0.01	0.022	0.012
5	0.005	0.017	0.012
10		0.025	0.0125
10		0.020	0.010

completeness to prevent interference with the final colorimetric reading. Excessive amounts of calcium give too great a precipitate to be dissolved in the 6 N HCl, as mentioned above, while phosphoric acid decolorizes the aurin. The amounts of these elements found in the tissues, however, have not been large enough to cause this difficulty. The table also indicates the freedom from aluminum of the sulfuric acid and iron solution used.

Table II gives some checks on the method of digestion, showing the small amount of aluminum found after the reagents have been carried through the process, and the recovery of aluminum added before digestion. In these cases the same samples of tissue were employed and the two digestions carried on simultaneously.

Table III includes a few of the checks made on the final readings by adding aluminum to aliquots of each digestion. The recovery of the calculated total shows that there is no loss of aluminum during the process, and that the color formed by the unknown is addable to that of aluminum. Use of different amounts of the digestion material is a further proof that the determinations are quantitative.

SUMMARY.

A colorimetric method of estimating the minute amounts of aluminum, which may be found in the various body tissues and fluids, is described. The essential features of the method are: (1) the digestion of the tissues with a sulfuric-perchloric acid mixture, (2) the precipitation of the aluminum along with a very small amount of iron and the subsequent complete separation from the iron, and (3) the development of a color reaction upon the aluminum with the ammonium salt of aurin tricarboxylic acid. The method is adequate for estimating the aluminum present in tissues under various conditions; *i.e.*, amounts varying from less than 0.01 to 0.5 mg. per 100 gm. of tissue. It is not suited to amounts of aluminum exceeding 1.0 mg. per 100 gm. of tissue. Owing to the delicacy of the method only reagents of the very highest purity can be used, but with suitable reagents and careful manipulation the error of the method should not exceed 10 per cent.

BIBLIOGRAPHY.

1. Steel, M., *Am. J. Physiol.*, 1911, xxviii, 94.
2. Kahn, M., *Biochem. Bull.*, 1911–12, i, 235.
3. Balls, A. K., *Biochem. Bull.*, 1916, v, 195, and unpublished dissertation, Columbia University, New York, 1917.
4. Steel, M., *Biochem. Bull.*, 1916, v, 173.
5. Schmidt, C. L. A., and Hoagland, D. R., *J. Biol. Chem.*, 1912, xi, 387.
6. Referee Board of Consulting Scientific Experts, *U. S. Dept. Agric., Bull. 103*, 1914.
7. Schmidt, C. L. A., and Hoagland, D. R., *Univ. Calif. Pub. Path.*, 1919, ii, 215.
8. Smith, E. E., Aluminum compounds in food, New York, 1928.
9. Killian, J. A., unpublished analyses cited by Smith (8).
10. Gray, P. B., cited by Smith (8).
11. Atack, F. W., *J. Soc. Chem. Ind.*, 1915, xxxiv, 936.
12. Hammett, L. P., and Sottery, C. T., *J. Am. Chem. Soc.*, 1925, xlvii, 142.
13. Schantl, E., *Mikrochem.*, 1924, ii, 174.

THE INFLUENCE OF THE ADMINISTRATION OF ALUMINUM UPON THE ALUMINUM CONTENT OF THE TISSUES, AND UPON THE GROWTH AND REPRODUCTION OF RATS.

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Experiments have been carried out on rats for the purpose of studying the influence of aluminum administration upon the content of aluminum in the tissues, and upon growth and reproduction.

EXPERIMENTAL.

The animals used, a mixture of pied and albino rats, were fed the same stock diet.[1] No effort was made to select a diet free from aluminum, but rather an adequate normal diet. The controls received only the stock diet while the experimental animals received in addition 2 mg. of aluminum per rat daily in a small biscuit made of flour and water, sweetened with saccharin, and dried in an oven until quite hard. This amount of aluminum, in the form of potassium aluminum sulfate, represents an average of 8 mg. of aluminum per kilo of body weight, and is 2 to 3 times that which the Referee Board (1) considered a large amount. All aluminum diet rats were kept on this diet for at least 100 days

[1] The diet consisted of: cracked yellow corn; soy beans, cooked 30 minutes under 20 pounds steam pressure; NaCl and $Ca_3(PO_4)_2$; together with about 25 cc. of pasteurized milk, 0.4 gm. of raw liver, 0.2 gm. of powdered Yeast Foam Tablets, and 3 drops of cod liver oil per rat per day. The salts were added to the cooked beans, 1 tablespoonful of each to a quart of beans. Roughly 3 parts of corn to 1 of beans were fed in the amounts the animals would take each day. No exact measures were made of the milk, liver, or yeast, but amounts approximating the quantitites stated were fed to each animal. The cod liver oil was added to the milk. Fresh water was supplied daily.

before being sacrificed for analysis. After the first generation the young were raised, weaned, and at once put on the experimental diet, so that they received the aluminum-containing biscuits from the time of weaning. The animals were killed for analysis by bleeding under ether anesthesia.

TABLE I.

Control Rats.

Generation.	Tissue.	Animals.	Tissue digested.	Analyses.	Al found.	Average Al content.
			gm.		*mg. per 100 gm.*	*mg. per 100 gm.*
2nd	Liver.	6	44.5	3	0.21	
2nd	"	6	34.5	2	0.07	
3rd	"	3	20.0	3	0.23	
3rd	"	5	43.0	2	0.14	
4th	"	8	60.5	2	0.06	0.14
2nd	Heart and tongue.	12	18.0	2	0.29	
3rd	" " "	5	8.0	1	0.11	
4th	" " "	8	12.0	1	0.09	0.16
4th	Thyroid.	8	7.0	1	0.18	0.18
2nd	Adrenal and kidney.	12	17.0	3	0.15	0.15
2nd	Spleen.	12	9.0	3	0.29	0.29
2nd	Lung.	12	12.0	3	0.00	
4th	"	8	12.0	1	0.13	0.06
2nd	Brain.	12	15.0	3	0.00	
4th	"	8	13.0	1	0.09	0.05
1st	Fetal young.	10	30.0	3	0.33	0.33
2nd	Young rat.	1	9.0	3	0.14	
2nd	" "	4	17.5	2	0.23	
2nd	" "	5	17.0	3	0.33	
2nd	" "	1	12.0	2	0.10	0.20
3rd	Blood.	3	21.0	2	0.05	
3rd	"	5	35.5	2	0.07	
4th	"	8	65.0		0.06	0.06

Aluminum Content of Tissues of Rats.

In making the analyses it was found necessary to combine the same tissues from animals in the same group in order to have sufficient material for satisfactory analytical work. The estimations of the aluminum were made by the method described in the preceding paper (2). The results obtained on the rats on the control

TABLE II.

Rats on Aluminum Diet.

Generation.	Tissue.	Animals.	Tissue digested.	Analyses.	Al found.	Average Al content.
			gm.		*mg. per 100 gm.*	*mg. per 100 gm.*
1st	Liver.	4	33.0	2	0.19	
1st	"	3	29.5	2	0.30	
2nd	"	4	25.0	2	0.23	
2nd	"	3	22.0	3	0.23	
3rd	"	12	101.0	4	0.11	
4th	"	8	63.5	2	0.12	0.19
1st	Heart.	7	7.5	1	0.24	
2nd	"	7	6.0	2	0.23	
3rd	"	12	11.0	1	0.12	0.20
2nd	Tongue.	7	5.5	2	0.00	
3rd	"	12	9.0	1	0.10	0.05
4th	Heart and tongue.	8	13.0	1	0.12	0.12
3rd	Thyroid.	12	11.0	1	0.21	
4th	"	8	7.5	1	0.16	0.19
1st	Kidney and adrenal.	7	13.0	1	0.00	
2nd	" " "	7	11.0	3	0.00	
3rd	" " "	12	21.5	1	0.10	0.03
1st	Spleen.	7	7.5	1	0.10	
2nd	"	7	6.0	2	0.00	
3rd	"	12	10.0	1	0.05	0.05
1st	Lung.	7	10.0	1	0.00	
2nd	"	7	10.0	2	0.00	
4th	"	8	12.5	1	0.14	0.05
1st	Brain.	7	11.5	1	0.39	
2nd	"	7	9.0	2	0.00	
3rd	"	12	21.0	1	0.15	0.18
3rd	Fetal young.	6	33.5	3	0.00	
4th	" "		23.0	1	0.09	0.05
2nd	Young rat.	1	13.0	1	0.15	
3rd	" "	5	23.0	3	0.06	
3rd	" "	5	24.0	1	0.12	
3rd	" "	6	22.0	2	0.14	
3rd	Blood.	12	106.0	3	0.00	
4th	"	8	67.5	3	0.01	0.00
1st	Testes.	4	11.0	1	0.36	
2nd	"	3	8.5	3	0.00	
3rd	"	5	13.0	1	0.12	
4th	"	4	11.0	1	0.09	0.14

diet are presented in Table I. There is considerable variation between the different analyses made, but it is believed that the averages furnish a fairly reliable index of the amounts of aluminum to be found in the different tissues. Similar determinations on the aluminum diet rats are given in Table II. There are no outstanding differences. In many cases the variations within the group are greater than those between the groups. However, the livers of the diet animals do run slightly higher in the maximum and minimum figures as well as in the average. The same is true of the brain tissue. Concerning other tissues no conclusions can be drawn.

TABLE III.

Rats on Aluminum-Free Diet.

Group No.	Tissue.	Animals.	Tissue digested.	Analyses.	Al found.	Average Al content.
			gm.		*mg. per 100 gm.*	*mg. per 100 gm.*
1	Liver.	3	23.5	2	0.13	
2	"	5	51.5	2	0.03	0.08
2	Heart and tongue.	5	10.5	1	0.06	0.06
2	Kidney and spleen.	5	16.0	1	0.07	0.07
2	Lung.	5	8.0	1	0.09	0.09
2	Brain.	5	9.5	1	0.20	0.20
2	Blood.	5	50.0	3	0.02	0.02

Since our experiments were completed a paper has appeared by Flinn and Inouye (3) in which they discuss the distribution of aluminum, copper, nickel, tin, and zinc in the various organs of the body when these metals are given in the food. Rats were used as the experimental animal and received 2 mg. of the metal a day. The analytical figures which they have given appear to be somewhat higher than those which we have found. It is difficult to compare their findings with our data, however, in view of the fact that in most cases their figures are given in mg. per rat instead of mg. per 100 gm. of tissue. They make no mention of the analytical methods which they employed.

A few rats, raised by Dr. Amy L. Daniels of the Child Welfare Research Station, on a diet as free from aluminum as possible, were analyzed. The results, given in Table III, show small amounts

of aluminum still present, indicating that it is either retained in the body or that it is very difficult to exclude from the food. The amount found in the brain in this case seems out of harmony with the other tissues and therefore may be open to question.

In order to study the effects and fate of aluminum when present in the body, several rats were injected intraperitoneally with 4 mg. of aluminum in the form of the potassium aluminum sulfate. No ill effects were observed, the animals continuing to gain normally and function as before. Analyses of the tissues and excretions, given in Table IV, show in particular a marked increase in the aluminum content of the liver, with elimination by the intestines

TABLE IV.

Rats Receiving Aluminum by Intraperitoneal Injection.

Material.	Amount digested.	Al found.	Al in sample used.	Days after injection of 4 mg. of Al.
	gm.	*mg. per 100 gm.*	*mg.*	
1 liver.	11.0	9.20	1.01	2
1 "	10.5	7.25	0.76	5
Blood and organs.	31.5	0.29	0.09	2
" " "	32.0	0.26	0.08	4
Feces.	7.5	9.73	0.73	9 day output.
"	2.0	10.00	0.20	3 day output, 2nd to 5th.
	cc.			
Urine.	31	0.32	0.10	3 day output, 2nd to 5th.

and probably to a small extent by the kidneys. It was not possible to rule out completely the contamination of the urine with the feces. These findings indicate that aluminum can be transported in the body, apparently by the blood stream. The large amount of aluminum in the liver would suggest its excretion into the intestines in the bile.

Influence of Aluminum upon Growth and Reproduction.

During the course of this work four generations of animals were raised, and the fifth weaned. Growth in both the control and

aluminum-fed groups was above Donaldson's (4) normal for male and female. In Fig. 1 are given the composite curves entirely typical of the second, third, and fourth generations. Since the

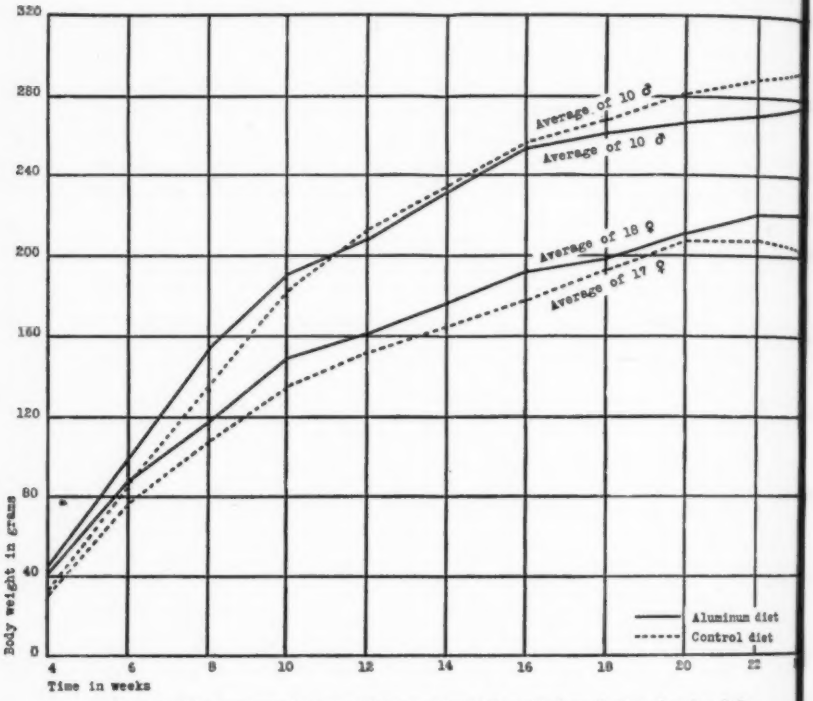

FIG. 1. The composite growth curve includes all the adult animals of the second, third, and fourth generations, distributed as follows: control group, second generation 4 males, 8 females; third generation 4 males, 6 females; fourth generation 2 males, 3 females; experimental group, second generation 3 males, 9 females; third generation 4 males, 6 females; fourth generation 3 males, 3 females. In addition to the stock diet each experimental rat received 2 mg. of aluminum per day.

first generation is not included, all the experimental animals represented were subject to the effect of the aluminum diet from the time of their conception. The only manifestation brought out by the curves, however, is a slightly more rapid initial growth

with the aluminum-fed stock. It is of interest in this connection that as early as 1913 Osborne and Mendel (5) reported significant traces of aluminum, fluorine, and manganese in milk and found that when traces of these elements were added to their inorganic salt mixture better growth was obtained. Daniels and Hutton

TABLE V.

Influence upon Reproduction of Addition of Aluminum to Diet.

Diet.	No. of females.	Average age.	Young born.	Average litter.	Per cent raised.
			First litters.		
		days			
Control	16	106	86	5.4	66
Aluminum	23	104	150	6.5	40
			Second litters.		
Control	3		25	8.3	64
Aluminum	6		56	9.3	78

TABLE VI.

Influence of Addition of Aluminum to Diet upon Excretion of Feces and Urine.

Diet.	No. of rats.	Average weight.	Days of collection.	Feces.		Urine.	
				Amount collected.	Daily average.	Amount collected.	Daily average.
		gm.		*gm.*	*gm.*	*cc.*	*cc.*
Aluminum	4	272	7	35.5	1.27		
Control	4	281	7	34.5	1.23		
"	4	212	2	8.5	1.06		
Aluminum	1	340	2	4.0	2.00		
Control	4	212	2			37	4.6
Aluminum	4	234	2			43	5.4
Control	4	226	2			43	5.4
Aluminum	4	256	2			34	4.3

(6) have shown that the addition to milk of these elements present in milk in low concentration, *viz.* aluminum, fluorine, and manganese, together with silicon, seem to be essential to satisfactory reproduction, an observation which has been confirmed by Mitchell and Schmidt (7). In view of our observations on the influence of

aluminum on growth (Fig. 1), the following statement of Hubbell and Mendel (8) regarding zinc is interesting; *viz.*, "The slightly favorable effect of adding 0.02 mg. was more evident with the females than with the males."

Comparisons on reproduction are made in Table V. Free intercourse was permitted until after the birth of the first litters, which came quite early. These were of normal size, the number still-born was almost negligible, and the percentage raised fairly good for the first litters.

Marked improvement is shown in the second litters, though due to lack of space fewer of these could be permitted. It is evident, however, that the diet was not the cause of the low percentage reared in the first litters, since such marked improvement was made during its continuation. One female, of the second generation, successfully raised eight young out of ten after 326 days on the aluminum diet, indicating no cumulative effect.

A comparison of the urinary and fecal excretions is shown in Table VI. The daily averages are almost the same when the collections were made over the same periods of time from males of about the same size and age. The stools were always formed, and no evidence of diarrhea was noted at any time. Similarly the urine was normal, no proteinuria being observed.

No abnormalities were noted grossly at autopsy and all animals appeared healthy. The animals having received the intraperitoneal injections showed no evidence of it. That their bodily functions were unimpaired is shown by the fact that they not only continued to grow at a normal rate, but were bred and bore young as before.

SUMMARY.

The aluminum content of the tissues of rats has been determined on four groups of animals: (1) on a control diet, (2) on a diet with a high aluminum content, (3) on an aluminum-free diet, and (4) following the intraperitoneal administration of aluminum.

Minute traces of aluminum are present in the tissues normally and these show only a slight increase on a diet containing considerable aluminum. Aluminum persists in the tissues even on an aluminum-free diet.

Following intraperitoneal administration, aluminum is found

in increased amounts throughout the body, the largest amounts apparently being in the liver. It appears to be excreted chiefly by way of the intestine.

For the liver the average findings of aluminum per 100 gm. of tissue were: (1) control diet 0.14 mg., (2) aluminum diet 0.18 mg., (3) aluminum-free diet 0.08 mg., and (4) aluminum administered intraperitoneally 8.22 mg.

Observations have been made covering four generations of rats receiving 2 mg. of aluminum in the form of potassium aluminum sulfate per rat daily in addition to the stock diet. The growth curves of these animals compare well with those of the controls, the only difference being that the aluminum-fed rats show a slightly greater initial growth. So far as could be ascertained the addition of aluminum to the diet was without other influence.

Addendum.—Since the present study was completed and submitted for publication, a paper has appeared by McCollum, Rask, and Becker (9) on a study of the possible rôle of aluminum compounds in animal and plant physiology, in which the rat was employed as the experimental animal. They noted no deleterious action of aluminum compounds upon growth, reproduction, or general well being of the animals. They employed the spectrographic method to estimate aluminum, but obtained essentially negative results on the tissues of both the control and aluminum diet rats.

BIBLIOGRAPHY.

1. Referee Board of Consulting Scientific Experts, *U. S. Dept. Agric., Bull. 103*, 1914.
2. Myers, V. C., Mull, J. W., and Morrison, D. B., *J. Biol. Chem.*, 1928, lxxviii, 595.
3. Flinn, F. B., and Inouye, J. M., *J. Am. Med. Assn.*, 1928, xc, 1010.
4. Donaldson, H. H., The rat, Philadelphia, 2nd edition, 1924.
5. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1913, xv, 311.
6. Daniels, A. L., and Hutton, M. K., *J. Biol. Chem.*, 1925, lxiii, 143.
7. Mitchell, H. S., and Schmidt, L., *J. Biol. Chem.*, 1926, lxx, 471.
8. Hubbell, R. B., and Mendel, L. B., *J. Biol. Chem.*, 1927, lxxv, 567.
9. McCollum, E. V., Rask, O. S., and Becker, J. E., *J. Biol. Chem.*, 1928, lxxvii, 753.

THE INFLUENCE OF THE ADMINISTRATION OF ALUMINUM UPON THE ALUMINUM CONTENT OF THE TISSUES OF THE DOG.

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In the study of the absorption of aluminum from the alimentary tract reported in the preceding paper (1) the rat was employed as the experimental animal. Owing to its small size and to the minute amounts of aluminum found to be present in animal tissues, the rat did not prove to be an ideal animal for this work, despite the very great delicacy of the analytical method used. For this reason a second study was undertaken on dogs.

The dog was the experimental animal employed in the experiments conducted by Steel (2), Kahn (3), and Balls (4) in Gies' laboratory to which reference has already been made (5). Although their analytical methods were scarcely adequate to determine the very small amounts of aluminum present, still they constitute the most serious attempt of the time to answer the questions raised. In general it would appear, however, that their analytical data are quite too high, probably by reason of the inadequacy of the methods employed. Steel (2) carried out eleven experiments in which from 50 to 200 mg. of aluminum were administered to dogs either in the form of potassium aluminum sulfate or in baking powder biscuits. After 3 hours, blood was withdrawn and analyzed by the method of the Association of Official Agricultural Chemists, slightly modified (6) and found to contain 0.1 to 0.8 mg. of Al per 100 gm. of blood.[1] From this he concluded that "aluminum in comparatively large amounts promptly passed into the blood." A little later Kahn (3) conducted experiments on three dogs to which from 15 to 25 mg. of Al were administered daily in baking powder biscuits, employing the same analytical methods as Steel. He found as high as 11 mg. of Al per 100 gm. in bile and pancreas in individual instances with an average of 0.7 mg. of Al per 100 gm. of blood and 1.9 mg. per 100 gm. of liver. He concluded that aluminum passes in considerable

[1] When the data in the literature have been given in terms other than aluminum they have been recalculated.

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amounts into the blood, accumulates to some extent in various parts of the body, and is excreted partly in both the bile and the urine. About 6 years later Balls (4) carried out analyses on the tissue of normal dogs and on dogs receiving aluminum, two animals being given aluminum in the form of the phosphate and two in baking powder biscuits. The Schmidt and Hoagland method (7) for aluminum was used. In the control group six samples of blood and a number of different tissues were analyzed, but with one exception, a sample of blood giving 0.05 mg. of Al per 100 gm. of blood, all of the results were regarded as being beyond the accuracy of the method. The blood of the aluminum-fed animals averaged about 0.1 mg. of Al per 100 gm., but wide variations were encountered in the aluminum content of the other tissues, the bile for example varying from 0.5 to 6.6 mg. of Al per 100 gm. Balls concluded: "Aluminum is absorbed by dogs from food containing aluminum phosphate, and from bread baked with alum baking powder. Much of this aluminum is speedily eliminated, some is retained, replacing part of the iron occurring normally in the tissues." In 1924 Killian (8) carried out tissue analyses with the Schmidt-Hoagland method on a dog which had received 190 mg. of aluminum daily for 7 weeks in the form of baking powder biscuits. The following results calculated in mg. of Al per 100 gm. of tissue were obtained: serum, 0.13; clot, 0.05; gallbladder and bile, 0.89; kidneys, 0.23; bones, 0.03. Before the aluminum diet was instituted the serum showed 0.04 mg. and the clot 0.06 mg. Although Killian did not regard the results dependable, owing to the minute amounts of aluminum found, his results show fair agreement with the figures reported in the present paper. Employing a modified Atack alizarin method Gray (9) obtained the following results on two normal dogs in individual or combined analyses calculated as mg. of Al per 100 gm. of tissue: blood 0.12, 0.14; kidney 0.60, 0.54; liver 0.50, 0.70; gallbladder 0.41; spleen 0.22; testicle 0.10; pancreas 0.10; bone marrow 0.72, figures which we believe too high in several instances.

EXPERIMENTAL.

The analytical method described in an earlier paper of this series (5) was applied to the tissues of normal dogs and dogs receiving aluminum. As a rule, when 40 gm. of tissue were available this amount was used for digestion, and color developments made on the equivalent of 12 gm. of tissue unless the aluminum content was known to be small, when larger amounts were employed. Most of the analyses on the normal dogs were made in triplicate, aluminum being added to one or two samples in each determination as a check on its recovery.

Aluminum Content of Tissues of Normal Dog.—With one exception the eight control dogs were obtained from the Department of Pharmacology after use in various experiments, in none of which

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inorganic salts were employed. The animals had been fed on scraps obtained from the hospital diet kitchen.

A summary of these results is presented in Table I. It will be noted that the highest content of aluminum was found in the liver, followed by the heart, kidney, and spleen. In two other normal dogs (Nos. 9 and 10) 6.1 gm. and 5.3 gm. of bile were analyzed and gave 0.33 and 0.38 mg. of Al per 100 gm. respectively.

Aluminum Content of Tissues of Dog on Diet Containing Added Aluminum.—The animals used in this series of experiments were all mature dogs, procured from various sources. They were of no particular breed and their previous history is unknown. All of

TABLE I.

Aluminum Content of Tissue of Normal Dog.

Dog No.	Liver.	Heart.	Kidney.	Spleen.
	Mg. Al per 100 gm. tissue.			
1	0.16	0.10	0.06	0.07
2	0.18	0.12	0.07	0.08
3	0.10	0.10	0.11	0.07
4	0.31	0.17	0.14	0.03
5	0.10	0.11	0.06	
6	0.12	0.10	0.07	0.04
7	0.08	0.02	0.03	0.05
8	0.17	0.17	0.12	0.14
Average....	0.15	0.11	0.08	0.07

Aluminum figures of 0.04 mg. and below are regarded as too small to be reliable.

the animals, with the exception of Dog 11 which was accidentally killed by a puncture of the coronary artery while blood was being taken, were kept on the diet for approximately 90 days. The diet consisted of a cooked mixture of hashed beef (with some bone), corn-meal, and oatmeal to which a little salt was added. In addition to this stock diet Dogs 11 to 16 first received a sufficient number of biscuits raised with baking powder containing aluminum to be equivalent to 230 mg. of aluminum daily. Dogs 17 and 18 received 7 gm. of aluminum phosphate in their food daily, the aluminum phosphate being thoroughly incorporated in the stock diet, the amount of which was always less than the amount the

dogs consumed at one feeding. After the food containing the aluminum phosphate was eaten additional stock diet was given to satisfy each animal. All animals were fed once daily at 11 a.m. The animals ate well, were always healthy, and appeared to thrive on the diet.

At the completion of the diet period, the animals were killed by bleeding from the femoral artery while under ether anesthesia.

TABLE II.

Aluminum Content of Tissues of Dogs Receiving Aluminum.

Dog No. Weight, *kg*	11 8.5	12 14	13 14.3	14 15	15 11	16 17.6	17 16.2	18 15	Aver-age.
Tissue or fluid.	Mg. Al per 100 gm. tissue or fluid.								
Blood			0.04	0.17	0.04	0.04	0.04	0.04	*
Bile		0.39	0.28	0.16	0.46	0.33	0.50	0.33	0.35
Liver	0.35	0.33	0.19	0.20	0.12	0.33	0.25	0.42	0.27
Intestine	0.43				0.27	0.18	0.32	0.35	0.31
Stomach	0.25								
Kidney	0.13	0.03	0.12	0.10	0.04	0.09	0.04	0.04	0.07
Urine			0.01	0.01		0.01		0.02	*
Heart		0.07	0.10	0.14	0.25	0.15	0.12	0.08	0.13
Muscle		0.21	0.10	0.13	0.10	0.12	0.05	0.17	0.12
Brain		0.18	0.04	0.04	0.05	0.09	0.12	0.16	0.10
Pancreas		0.12	0.13	0.10	0.12	0.11	0.08	0.04	0.10
Spleen	0.15		0.04	0.04	0.04	0.06	0.04	0.17	0.08
Lungs				0.08	0.12	0.17	0.04	0.08	0.10
Bone			0.04	0.04	0.04	0.04	0.04	0.04	*
Testes	0.14								

Dogs 11 to 16 received 0.23 gm. of aluminum daily for 90 days (excepting Dog 11 which was accidentally killed earlier) in biscuits raised with a baking powder containing aluminum, while Dogs 17 and 18 had 1.55 gm. of aluminum in the form of $AlPO_4$ daily incorporated in their food.

* Figures of 0.04 mg. and below are regarded as too small to be reliable.

No food was given the animals for 26 to 32 hours preceding the bleeding. The tissues were handled very carefully to prevent any contamination. The urine analyzed was obtained by catheter. The averages of duplicate analyses are summarized in Table II. Although the intake of aluminum in these animals was comparatively high, 17 mg. of Al per kilo for Dogs 11 to 16 and 105 mg. per kilo for Dogs 17 and 18, still there is relatively

little difference in the aluminum content of the tissues from that found in the control group, except in the case of the liver, where the average is 0.27 mg. instead of 0.15 mg. of Al per 100 gm. The figures for the bile, however, are the same for both the control dogs, Nos. 9 and 10, and Dogs 12 to 18 on the aluminum diet, namely 0.35 mg. per 100 gm. It is of some interest that the heart and muscle appear to contain slightly more aluminum than the brain, pancreas, or lungs, while the kidney contains less, and the figures obtained for the urine are too small to attach any significance to them.

Absorption and Excretion of Aluminum.—Several experiments have been carried out dealing with the absorption and excretion of aluminum. The absorption of aluminum salts was studied in one experiment from intestinal loops, two experiments were conducted on the time of elimination of injected aluminum, while two experiments were made to ascertain the excretion of injected aluminum by way of the bile and the urine.

For the intestinal loop experiments a dog weighing 10 kilos (Dog 19) was employed. A section of the intestine about 18 inches long was irrigated with warm physiological saline until the return was perfectly clear, then divided into two loops with the aid of broad tape ligatures so as to interfere as little as possible with the mesenteric circulation. Cannulæ were inserted through small incisions, tied in place, and 25 cc. of a warm solution of potassium aluminum sulfate in physiological saline injected. Unfortunately a part of the solution in the second loop was lost by a peristaltic contraction before it could be closed. The loops were replaced in the abdomen and the wound closed. After 3 hours the loops were removed. The aluminum had apparently precipitated on the mucosa in curdy lumps. The first loop was thoroughly cleaned and the aluminum content determined by the Schmidt-Hoagland method (7). The loop was found to contain 130.5 mg. of aluminum in comparison with 130.2 and 129.9 mg. determined in 25 cc. of the solution with the same method, thus indicating a complete lack of absorption.

The aluminum content of the tissues of two dogs was studied after the intravenous and intraperitoneal injection of potassium aluminum sulfate. The data are presented in Table III. It will be observed that a marked retention of aluminum existed after 8

days and that considerable aluminum was present in Dog 21 after the lapse of 34 days. The aluminum appears to have been well distributed, although the largest amounts were found in the abdominal muscle and skin. In harmony with the findings reported in the preceding paper (1) the figures for the aluminum content of the liver are very high. However, the aluminum

TABLE III.

Analysis of Tissue after Injection of Aluminum.

Tissue or fluid.	Dog 20.			Dog 21.		
	Amount digested.	Aliquot analyzed.	Al found.	Amount digested.	Aliquot analyzed.	Al found.
	gm.	*gm.*	*mg. per 100 gm.*	*gm.*	*gm.*	*mg. per 100 gm.*
Blood	54.0	5.4	0.18	40	12.0	0.06
Bile	12.5	12.5	0.32	9	9.0	0.44
Liver	40.0	1.6	2.25	40	3.2	0.87
Intestine	40.0	1.6	1.87			
Kidney	40.0	1.6	1.56	40	6.0	0.33
Heart	40.0	1.6	2.00	40	6.0	0.60
Abdominal muscle	40.0	0.8	4.38	20	2.0	1.15
" skin	31.0	0.6	5.64	20	2.0	0.90

Dog 20, a female weighing 8.6 kilos, was given intravenously 25 cc. of warm (37°) sterile physiological saline containing 5 mg. of Al in the form of potassium aluminum sulfate on Nov. 15 and 16, 1927, and intraperitoneally on each of the 12 following days, a total of 70 mg. of Al. Beyond the slight irritation from repeated punctures no ill effects were observed. The animal was killed by bleeding 8 days after the last injection.

Dog 21, a female weighing 10.6 kilos, was given 70 mg. of Al, the first 5 mg. injection being made intravenously on Nov. 16, 1927, while the remaining thirteen injections were given intraperitoneally on successive days. The animal showed no ill effects from the injections but 1 month later, possibly due to overheating and drafts in the animal house, developed a respiratory infection and died 3 days later, 34 days after the last injection.

content of the bile is practically normal, and thus furnishes no evidence of an increased excretion of aluminum in the bile. Unfortunately the specimens of urine were lost. In comparison with the observations on the diet animals it is significant that here appreciable amounts of aluminum were present in the blood of both dogs. The large amount of aluminum still present in the

body after periods of 8 and 34 days indicates a rather slow elimination of aluminum when once present in the body, and furnishes little support to the view that appreciable amounts of aluminum are absorbed from the alimentary tract.

In an attempt to demonstrate the excretion of injected aluminum in the bile two experiments have been performed. Two unfed female dogs, Dog 22 weighing 10.4 kilos, and Dog 23 weighing 9 kilos, were given 1.5 gm. of urethane per kilo of body weight. As soon as the urethane had taken effect, ether was given, a tracheal cannula inserted, and the gallbladder exposed by a median incision of the abdominal wall. The gallbladder was ligated and the common bile duct cannulated from the lumen of the intestine.

TABLE IV.

Influence of Intravenous Administration of Aluminum upon Aluminum Content of Bile and Urine.

Animal No.	Fluid.	Fluid obtained and digested.	Aliquot used.	Al found.
		gm.	*gm.*	*mg. per 100 gm.*
22	Gallbladder bile.	5.3	5.3	0.38
	Cannula "	7.5	2.25	0.44
	Urine.	15.0	4.5	0.67
23	Gallbladder bile.	Lost.		
	Cannula "	14.6	14.6	0.23
	Urine.	20.0	5.0	0.40

The cannula bile was collected over a period of 3½ hours in Dog 22 and 4½ hours in Dog 23.

30 cc. of 0.35 per cent hydrochloric acid were injected into the intestine posterior to the opening of the bile duct to stimulate the flow of bile, and the bile collected. The femoral vein was next exposed and 100 cc. of warm (37°) physiological saline containing 10.84 mg. of aluminum as aluminum chloride were injected. A catheter was also inserted into the urinary bladder and the urine collected.

At the close of the experiment the ligated gallbladder was removed and the contents analyzed as normal bile.

The data obtained are summarized in Table IV. It seems hardly proper to compare the cannula bile with the bladder bile since the latter is normally 6 to 10 times more concentrated.

However, in Dog 22 the cannula bile is found to be slightly richer in aluminum. The figures for the urine are so high that they would suggest the kidney as one path for the excretion of aluminum, at least when appreciable amounts are present in the blood.

DISCUSSION.

Traces of aluminum are present in the tissues of the normal dog. Of the tissues analyzed, heart, kidney, spleen, and liver, the latter contained the largest amount, averaging in eight animals 0.15 mg. of Al per 100 gm. The administration of relatively large amounts of aluminum has practically no influence upon the aluminum content of the heart, kidney, spleen, and bile, although in the case of the liver there appears to be a very slight but definite influence, the average figure for eight animals being 0.27 mg. of Al per 100 gm. The figures obtained for the aluminum content of the blood are so small as to be within the limits of experimental error of the method.

When a small amount of aluminum (70 mg.) was administered to dogs intravenously and intraperitoneally in 5 mg. daily portions, it apparently remained in the tissues for a long time after the last injection, since very high figures were found at the end of 8 days, and after 34 days the amount was still 3 or more times that found in the aluminum diet animals. In the one experiment in which the question of absorption of aluminum from an intestinal loop was studied complete recovery of aluminum was obtained.

The data presented in this paper, we believe, speak strongly against the absorption of more than traces of aluminum from the alimentary tract. If this view is incorrect, it is hard to comprehend why small amounts of injected aluminum should be retained in the tissues for a long time, when very large quantities given by mouth daily (45 to 300 times as much) have only a very slight influence. In other words, if aluminum is poorly eliminated when parenterally introduced, it is illogical to believe that when orally introduced it should be absorbed and rapidly eliminated. To be sure we have observed an appreciable excretion in the urine when aluminum chloride was introduced intravenously (see Table IV), but if a significant amount of ingested aluminum were normally eliminated in this way, this fact would not have been overlooked by the Referee Board (10) and by Schmidt and

Hoagland (11), nor would they have obtained complete recovery in the feces of the ingested aluminum. We do not feel, therefore, that our observations lend any support to the contention of Steel (2), Kahn (3), and Balls (4), that considerable quantities of aluminum are absorbed from the digestive tract of the dog when aluminum is ingested in the food.

There are three possible paths for the elimination of aluminum when it is present in the body tissues and fluids: the bile, the urine, and the skin. Kahn (3) concluded that aluminum was excreted to some extent in both the bile and the urine, which is probably correct. Our data do not appear to give a definite answer as to which is the chief path of excretion, and more work is needed on this point. That some aluminum may be excreted in the urine after the injection of aluminum would seem evident from the data presented in this and the preceding paper. If the kidney is the most important path for the excretion of aluminum it does not retain the aluminum long as the aluminum content of this tissue is relatively low. We have been inclined to regard the excretion through the bile as the most important owing to the higher concentration of aluminum in the liver and the bile than in the other tissues and fluids, but the fact that the dogs receiving aluminum in their food or by injection showed essentially the same concentration of aluminum in the bile as the normal dogs does not strengthen this view.

The dogs receiving aluminum by intraperitoneal injection showed the highest content of aluminum in the abdominal muscle and skin, but this may well have been influenced by the site of administration.

SUMMARY.

Aluminum is normally present in such tissues of the dog as the spleen, kidney, heart, and liver in amounts varying from 0.07 to 0.15 mg. per 100 gm., the largest amounts being present in the liver.

After the daily ingestion of 0.23 and 1.55 gm. of aluminum (six and two animals respectively) for 3 months, there was practically no change in the aluminum content of these same tissues, with the exception of the liver, where the amount averaged 0.27 mg. per 100 gm. The aluminum content of the bile of these

animals and two control animals averaged 0.35 mg. in both groups. The figures obtained for the aluminum content of the blood of the aluminum diet animals were so small as to be within the limits of experimental error of the method.

When 5 mg. of aluminum were administered parenterally to two dogs daily for 2 weeks, a marked increase in the aluminum content of the tissues was found 8 and 34 days after the administration of aluminum had been discontinued, indicating that when aluminum is present in the tissues it is slowly excreted.

These data lead to the conclusion that when aluminum compounds are administered orally to dogs the absorption of the aluminum is very slight.

The excretion of aluminum when present in the body tissues and fluids is discussed.

BIBLIOGRAPHY.

1. Myers, V. C., and Mull, J. W., *J. Biol. Chem.*, 1928, lxxviii, 605.
2. Steel, M., *Am. J. Physiol.*, 1911, xxviii, 94.
3. Kahn, M., *Biochem. Bull.*, 1911–12, i, 235.
4. Balls, A. K., *Biochem. Bull.*, 1916, v, 195, and unpublished dissertation, Columbia University, New York, 1917.
5. Myers, V. C., Mull, J. W., Morrison, D. B., *J. Biol. Chem.*, 1928, lxxviii, 595.
6. Steel, M., *Biochem. Bull.*, 1916, v, 173.
7. Schmidt, C. L. A., and Hoagland, D. R., *J. Biol. Chem.*, 1912, xi, 387.
8. Killian, J. A., unpublished analyses cited by Smith, E. E., Aluminum compounds in food, New York, 1928, 228.
9. Gray, P. B., cited by Smith, p. 229.
10. Referee Board of Consulting Scientific Experts, *U. S. Dept. Agric., Bull. 103*, 1914.
11. Schmidt, C. L. A., and Hoagland, D. R., *Univ. Calif. Pub. Path.*, 1919, ii, 215.

THE ALUMINUM CONTENT OF HUMAN AUTOPSY TISSUE.

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Comparatively little attention appears to have been given to the aluminum content of human tissue, although a few analyses have been reported by Gonnermann (1, 2) and Keilholtz (3), the former obtaining relatively high values and the latter negative results. We have thought it desirable to present the few observations which we have made on human tissue at this time. On several occasions attempts have been made to estimate the aluminum content of human blood (4), on samples of from 5 to 30 cc., but the amounts of aluminum present appear to be too small to estimate satisfactorily.

The method previously described (5) has been applied to human autopsy material, kindly furnished us at the Pathological Laboratory of the City Hospital. Our analytical data are presented in Table I. As will be noted, the magnitude of the figures is quite comparable with those found in the rat (6) and dog (7). In contrast with the control analyses on the rat and dog, however, the figures for the liver appear to be much lower, whereas the aluminum content of the brain and heart is considerably higher. We do not wish to draw any definite conclusions from the limited number of observations recorded on human tissue, but we have thought it desirable to present the data available in conjunction with our findings on the rat and dog.

BIBLIOGRAPHY.

1. Gonnermann, M., *Biochem. Z.*, 1918, lxxxviii, 401.
2. Gonnermann, M., *Z. physiol. Chem.*, 1920, cxi, 32.
3. Keilholtz, A., *Pharm. Weekbl.*, 1921, lviii, 1482.
4. Mull, J. W., Morrison, D. B., and Myers, V. C., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 476.

5. Myers, V. C., Mull, J. W., and Morrison, D. B., *J. Biol. Chem.*, 1928, lxxviii, 595.
6. Myers, V. C., and Mull, J. W., *J. Biol. Chem.*, 1928, lxxviii, 605.
7. Myers, V. C., and Morrison, D. B., *J. Biol. Chem.*, 1928, lxxviii, 615.

TABLE I.

Aluminum Content of Human Autopsy Tissue.

Case No.	Diagnosis.	Brain.	Heart.	Liver.	Gall bladder and bile.	Kidney.	Spleen.
		Mg. Al per 100 gm. tissue.					
1	Peritonitis		0.27	0.05	0.05	0.02	
2	Acute alcoholism	0.14					
3	Bronchopneumonia	0.45	0.13	0.10		0.06	0.05
4	Diphtheria			0.05	0.03		
5	Advanced pulmonary tuberculosis	0.36			0.10	0.10	
6	Carcinoma of esophagus		0.14	0.09	0.17	0.06	0.13
7	Pulmonary tuberculosis		0.25		0.01		
8	Tubercular bronchopneumonia		0.23	0.12		0.20	0.12
9	Typhoid ulceration	0.07					0.03
10	Cardiorenal disease	0.20					
11	Acute splenic hyperplasia, *etc.*	0.22		0.07			
12	Heart failure	0.12	0.22	0.14		0.05	
13	" " pulmonary tuberculosis	0.35	0.29			0.34	0.07
14	Bronchopneumonia		0.11				0.05
No. of specimens		8	8	7	5	7	6
Average		0.25	0.21	0.08	0.07	0.10	0.07

ON THE SEPARATION OF HISTIDINE AND ARGININE.

IV. THE PREPARATION OF HISTIDINE.*

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Histidine was discovered simultaneously by Kossel (1) and by Hedin (2). Kossel first obtained it by precipitation with mercuric chloride from the alkaline solution containing the products of hydrolysis of the protamine, sturine. Hedin isolated it from the precipitate formed when the base fraction from hydrolyzed protein was treated with silver nitrate and then with ammonia until a maximum precipitation occurred. Both methods were subsequently further developed by Kossel and his associates, but for purposes of large scale preparation the mercuric chloride method has been almost universally employed. Fränkel (3) in 1903 suggested that hemoglobin was the most suitable source of histidine and described its preparation by precipitation with mercuric chloride from a solution maintained alkaline with sodium carbonate. Modifications of this procedure have been repeatedly described (4–8), but it is clear, from the pains that have been taken by different workers to define the exact conditions for success, that much difficulty has been encountered in obtaining good yields of a pure product.

While we have, as yet, had no occasion to study in detail the precipitation of amino acids by mercuric chloride in alkaline solution, the fact that lysine may be precipitated in this way (9, 10) indicates that the procedure is not necessarily as selective for histidine as has been supposed. We have therefore felt that

* The expenses of this investigation were shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington, D. C.

some of the other precipitants for histidine should be investigated with the object of developing a method for the preparation of this base which might be less subject to unaccountable variations in the quantity and quality of the product ultimately obtained.

Hedin's method for precipitating histidine by the use of silver was developed by Kossel and Kutscher (11) into a quantitative method of analysis and the further step of precipitating the histidine by Hopkins' mercuric sulfate reagent, subsequently advocated by Kossel and Patten (12) and by Osborne, Leavenworth, and Brautlecht (13), added greatly to the accuracy of this method.

As we have pointed out in a previous paper (14) histidine is completely precipitated when an excess of silver ion is added to a protein hydrolysate and the reaction is brought to pH 7.0 by the addition of barium hydroxide. The convenience of a direct separation of histidine in this way from most of the other products of hydrolysis of the protein is apparent and, while silver precipitation at pH 7.0 is by no means highly selective, it appears that very little in addition to histidine that is so precipitated is subsequently thrown down by mercuric sulfate. The removal of the small proportion of impurity is easily effected by suitable treatment of the final product. This method is, in principle, similar to Kossel and Patten's (12) method for the determination of histidine, and the more nearly quantitative nature of the precipitations render it, in our estimation, more suitable for the preparation of this base than the customarily employed mercuric chloride-sodium carbonate method. By proper attention to details of technique the labor involved in the operations can be reduced to a minimum.

The general procedure is as follows: (1) The protein (hemoglobin or washed and coagulated blood cells) is hydrolyzed and the greater part of the acid removed. (2) An excess of silver oxide suspended in water is added, the acidity of the solution being maintained by the addition of sulfuric acid as required but any material excess should be avoided. (3) The solution is brought to pH 7.4 by the addition of barium hydroxide solution and the precipitate, which contains the histidine together with other amino acids, is filtered off and washed. (4) The precipitate is suspended in water, sulfuric acid sufficient to give a faint reaction to Congo

red is added, and hydrogen sulfide is then passed in until the precipitate is completely decomposed. The silver sulfide is centrifuged off and the solution is concentrated to remove hydrogen sulfide. (5) The solution is made to contain 5 per cent of sulfuric acid, treated with Hopkins' mercuric sulfate reagent in excess, and allowed to stand at least 24 hours. The precipitate is filtered off, washed with diluted reagent, and decomposed with hydrogen sulfide. The mercuric sulfide is removed, the solution is concentrated slightly to expel hydrogen sulfide and is then treated with sufficient of a solution of recrystallized barium hydroxide to bring the reaction to pH 7.2 and filtered. (6) The perfectly clear filtrate is, if necessary, boiled with a little norit and concentrated *in vacuo* until a part of the histidine has separated in crystalline form. The flask is removed from the still and the contents heated until all is again in solution, and approximately an equal volume of warm absolute alcohol is added. After the mixture has stood in ice overnight, crude histidine separates very completely and is filtered off and washed with 80 per cent and then with absolute alcohol. A second small crop may be obtained by concentration of the mother liquors and similar treatment.

If further purification is desired this material may be converted to the dichloride and crystallized and free histidine recovered as described below.

Discussion of the Procedure.

The best material for the preparation of histidine is hemoglobin which can be very easily prepared in crystalline form by the method of Ferry and Green (15). Failing this, crude blood cells are probably the most readily available and cheapest source. We have found it useful to prepare a stock of dry material. Fresh cattle blood (22 liters) was diluted with an equal volume of 1 per cent sodium fluoride solution and centrifuged. The serum was drawn off as closely as possible and the pulp of cells diluted with an equal volume of 0.9 per cent sodium chloride solution and again centrifuged. The pulp was then slowly poured into a large volume of boiling distilled water, the coagulum strained off, pressed, ground, and washed with liberal amounts of hot water, pressed, ground, and extracted with 80 per cent alcohol, and finally dried and ground to a fine powder. Yield 2800 gm., nitrogen 16.7 per

cent ash- and moisture-free. This material, while it contains appreciable amounts of serum proteins is largely hemoglobin and is an excellent source of histidine.

Hydrolysis of the protein may be effected either with 6 N hydrochloric acid or with 8 N sulfuric acid. For most of this work we have used hydrochloric acid, but serious losses of histidine may occur during the removal of silver chloride unless this is extracted with hot dilute hydrochloric acid and the extract subsequently worked over. We therefore recommend the use of sulfuric acid. If the barium sulfate is washed by decantation as described below, very little labor is required.

The single experiment in which hydrolysis was effected in an autoclave as described in a previous paper (14) was not satisfactory as it was found very difficult to secure a pure product. This method of hydrolysis is therefore not to be recommended for the preparation of histidine although further investigation may show how it may be improved.

The addition of an excess of silver oxide to the solution maintained slightly acid to Congo red or brom-phenol blue usually presents no difficulty unless the acidity is allowed to become too great. We have occasionally worked with solutions which failed to give the brown spot test with barium hydroxide although silver sulfate was separating in crystalline form on the walls of the vessel.

If such a solution is carefully treated with barium hydroxide solution until the reaction is only faintly acid to brom-phenol blue paper, it will usually show the presence of excess silver after the addition of a little further silver oxide.

We have not carried through this preparation using silver nitrate instead of the oxide. Previous experience with the nitrate has shown, however, that no difficulties are to be expected. The silver precipitate obtained under these conditions should be rather more thoroughly washed than is necessary when the oxide is used.

The precipitation at pH 7.4 instead of at pH 7.0, which we have previously recommended, is necessary when one is dealing with a protein rich in histidine since, otherwise, a little histidine escapes precipitation.

The precipitation with Hopkins' reagent calls for little comment. The solution should have a volume of about 1 liter for

each 10 gm. of histidine expected and such a solution usually requires about 1½ volumes of the reagent for complete precipitation. Mercuric sulfate solution should be added until a clear sample of the fluid gives no more precipitate when a little reagent is added and the sample is allowed to stand for some time. The amount of histidine left in the filtrate from such a precipitation is insignificant.

Although in his first paper on the subject Kossel showed that free histidine could be readily obtained in crystalline form, most workers have made use of the salts, particularly the mono- and dihydrochlorides. The ease with which the free base may be brought to crystallization at its isoelectric point (16) suggested that it might advantageously serve for isolation. Unfortunately the crude histidine so obtained is contaminated with monoamino acids among which tyrosine has been positively identified. The material usually contains approximately 25 per cent of nitrogen (theory 27.1 per cent) but for some purposes such a preparation, which is free from other bases, is sufficiently pure. In order to secure analytically pure histidine it is necessary to carry out the crystallization of a salt since, owing to the nature of the impurities, the recrystallization of the crude material is ineffective.

EXPERIMENTAL.

Hydrolysis.—500 gm. of dry coagulated blood cells were treated with 2.5 liters of 8 N sulfuric acid (567 cc. of concentrated sulfuric acid + 2100 cc. of water) and heated in a boiling water bath with frequent shaking for several hours. The material was then boiled for 30 hours and poured into about 15 liters of distilled water. Approximately 95 per cent of the calculated amount of crystallized barium hydroxide was weighed out (1 cc. of concentrated sulfuric acid requires 5.56 gm.), dissolved in boiling water, and added in a thin stream with constant stirring. The barium sulfate was allowed to settle and the clear fluid drawn off. The barium sulfate was washed twice by decantation with a liberal amount of water and finally centrifuged. The clear fluids were concentrated either in a large vacuum still, or by boiling in open dishes, to 6 liters. A small amount of silver oxide suspension was added and the trace of silver chloride (derived from the chloride contamination of the material employed) and any remaining barium sul-

fate removed by filtration through paper pulp. An excess of silver oxide was then added as shown by the formation of a buff-colored precipitate when a drop was treated with a drop of cold barium hydroxide solution, and the solution was brought to pH 7.4 by the addition of warm saturated barium hydroxide solution.[1] The precipitate was filtered off and thoroughly washed with water on the funnels. It was then suspended in water, disintegrated by brushing through silk bolting cloth, acidified with sulfuric acid, and treated with hydrogen sulfide on a shaking machine. The precipitate was removed and washed and the fluid concentrated to approximately 2.5 liters. Concentrated sulfuric acid to make 5 per cent was added and the histidine precipitated by the addition of 4.5 liters of Hopkins' reagent. After standing 2 days the precipitate was removed and washed, suspended in water, acidified with sulfuric acid, and decomposed with hydrogen sulfide. The mercuric sulfide was removed and washed and the solution concentrated. A solution of recrystallized barium hydroxide was added until the reaction was at pH 7.2. As it is inadvisable to return the test samples containing indicator to the main solution, the most economical method of adjusting the reaction is by comparison on a porcelain plate of the color of single drops to which a dilute solution of indicator has been added with drops of appropriate buffer solutions. A final test may be made on a small sample which has been centrifuged clear. The isoelectric point of histidine is not known with sufficient accuracy to necessitate closer control than can readily be obtained in this way.

The solution was filtered, concentrated *in vacuo* to approximately 500 cc., boiled with norit, and further concentrated until a copious separation of crystalline histidine occurred. The flask was removed from the still, heated until the histidine had redissolved, and the contents (180 cc.) were washed with a minimal amount of hot water into a beaker. An equal volume of hot absolute alcohol was added. Under these conditions histidine separates promptly and very completely when allowed to stand in ice overnight. A small second crop can be secured from the mother liquor.

Crude histidine may be prepared in this way in a yield of between 4 and 5 per cent of the blood cells hydrolyzed. It

[1] For additional details see Vickery and Leavenworth (14, 17).

contains a little tyrosine and probably other amino acids. A typical first crop weighing 21 gm. decomposed at 268–270° and was ash-free. This indicated a high degree of purity but its nitrogen content was 25.24 per cent (theory 27.10 per cent) and Millon's reaction was faintly positive. Recrystallization from 300 cc. of 50 per cent alcohol gave 16 gm. with a nitrogen content of 25.56 per cent, indicating very little purification although the material was uniform and well crystallized and decomposed as before at 268°. Evidently the decomposition point of histidine cannot be used as an index of purity.

Purification of Crude Histidine.—A sample of crude histidine weighing 26.7 gm. (N 24.4 per cent) was dissolved in 150 cc. of water with the aid of a little hydrochloric acid and boiled with norit. The solution was then concentrated *in vacuo* to 40 cc. and transferred, by the use of 40 cc. of concentrated hydrochloric acid, to a beaker. After it was chilled, 80 cc. of alcohol saturated with hydrogen chloride were added. Crystallization occurred at once and, after it had stood in ice 3 hours, 34 gm. of dihydrochloride were obtained by filtration on a sintered glass funnel and drying over lime *in vacuo*. This crop contained 17.69 per cent of nitrogen (theory 18.43 per cent) and was therefore still slightly impure. It was recrystallized from a mixture of 20 cc. of water, 20 cc. of concentrated hydrochloric acid, and 40 cc. of alcoholic hydrogen chloride, when 31 gm. with a nitrogen content of 18.35 per cent were secured. This is equivalent to 21 gm. of free histidine or 78 per cent of the crude histidine taken. When histidine dihydrochloride is crystallized under these conditions it is not worth while to attempt to secure second crops.

A small quantity of tyrosine was isolated from the mother liquor of this second crystallization of the histidine dichloride. The reagents were removed and a small amount of typical needles giving Millon's reaction and containing 8.0 per cent of nitrogen (theory 7.74 per cent), was secured by direct crystallization. The mother liquor of the tyrosine apparently contained a mixture of amino acids from which nothing definite could be separated.

The recovery of free histidine from the dihydrochloride may be conveniently effected as follows, although the yield leaves something to be desired. 20.3 gm. of dihydrochloride were dissolved in 200 cc. of water and heavy magnesium oxide was added to the

boiling solution until it was neutral to litmus. The solution was then promptly filtered. Further small amounts of magnesia were then added until the solution was slightly more alkaline than pH 7.2. The solution was boiled with norit, filtered, the reaction adjusted to pH 7.2 with dilute hydrochloric acid, and concentrated to 90 cc. An equal volume of absolute alcohol was added and, when the solution was chilled for 24 hours, 10.4 gm. of free histidine separated. The mother liquor on evaporation to 20 cc. and treatment as before with alcohol yielded 0.9 gm. more. Together these crops represent 81 per cent of the histidine in the dihydrochloride used. Once recrystallized the product was analytically pure.

Recovery of Silver.—Inasmuch as the use of ammonia for the recovery of silver residues is objectionable for a number of reasons, among them the possibility of the formation of explosive compounds, we have discarded the method described in a former paper (14) and are now making use of the method described by Robertson (18). We have found this perfectly satisfactory even when the silver chloride contains considerable barium sulfate. The mixture of metallic silver and barium sulfate, obtained by reduction with glucose, is heated in a large silica dish to drive off organic impurities and then warmed with dilute nitric acid until all the silver is dissolved. The barium sulfate may then be removed by filtration. We have also found it possible, although not particularly advantageous, to recover silver sulfide directly by this method without previously oxidizing it to sulfate with nitric acid. It is necessary to adhere to the concentration of sodium hydroxide solution advocated by Robertson and to use a grade of sodium hydroxide free from iron as otherwise the silver oxide ultimately prepared is apt to be contaminated with this metal.

SUMMARY.

A method for the preparation of histidine from hemoglobin, or from coagulated blood cells, is described, consisting of four steps: (1) hydrolysis of the protein, (2) precipitation of histidine as its silver compound at pH 7.4, (3) precipitation of histidine with Hopkins' mercuric sulfate reagent, (4) direct crystallization of crude histidine as free base at its isoelectric point. The product

may be finally purified, with little loss, by crystallization of the dihydrochloride. A yield of from 4 to 5 per cent of the dry blood cells is readily secured.

BIBLIOGRAPHY.

1. Kossel, A., *Z. physiol. Chem.*, 1896, xxii, 176.
2. Hedin, S. G., *Z. physiol. Chem.*, 1896, xxii, 191.
3. Frānkel, S., *Monatsh. Chem.*, 1903, xxiv, 230.
4. Knoop, F., *Beitr. chem. Physiol. u. Path.*, 1907, x, 111.
5. Brigl, P., *Z. physiol. Chem.*, 1910, lxiv, 337.
6. Jones, H. M., *J. Biol. Chem.*, 1918, xxxiii, 429.
7. Demjanowski, S., *Z. physiol. Chem.*, 1922, cxxii, 93.
8. Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1920, xliii, 521.
9. Vickery, H. B., *J. Biol. Chem.*, 1924, lxi, 117.
10. Winterstein, E., *Z. physiol. Chem.*, 1905, xlv, 77.
11. Kossel, A., and Kutscher, F., *Z. physiol. Chem.*, 1900, xxxi, 165.
12. Kossel, A., and Patten, A. J., *Z. physiol. Chem.*, 1903, xxxviii, 39.
13. Osborne, T. B., Leavenworth, C. S., and Brautlecht, C. A., *Am. J. Physiol.*, 1908, xxiii, 180.
14. Vickery, H. B., and Leavenworth, C. S., *J. Biol. Chem.*, 1927, lxxv, 115.
15. Ferry, R. M., and Green, A. A., in preparation.
16. Kanitz, A., *Z. physiol. Chem.*, 1906, xlvii, 476.
17. Vickery, H. B., and Leavenworth, C. S., *J. Biol. Chem.*, 1927, lxxii, 403.
18. Robertson, G. R., *J. Am. Chem. Soc.*, 1927, xlix, 2884.

THE RELATION OF COPPER TO THE HEMOGLOBIN CONTENT OF RAT BLOOD.*

PRELIMINARY REPORT.

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(Received for publication, May 19, 1928.)

In previous publications by the senior author (1–9) it has been shown that copper, manganese, and zinc are normal constituents of plants and animals, and that these elements occur in greater concentration in the livers of domestic animals than in any other organ. It has also been found that manganese, copper, and zinc occur in greatest concentrations in those organs of animals that have the greatest vitamin potency, and furthermore it has been shown that the livers of calves at the time of birth contain more copper than do the livers of more mature animals of the same species.

Porter (10) in 1875 published a text-book on chemistry in which he states that copper is a normal constituent of human blood. However, it has not been previously demonstrated that copper has an important function in red blooded animals, although numerous observations have been published from time to time which indicate that copper has a respiratory function in those animals that have colorless and blue blood.

The following experiment was performed to ascertain the copper content of the serum and cells of fresh blood of a mature domestic animal. 5 quarts of fresh blood were obtained from a normal 2-year-old heifer which was slaughtered for beef. The fresh, warm blood was shaken with glass beads to separate the fibrin and cells from the serum. The mixed mass was centrifuged

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to free further the serum from the cells and fibrin. The clear serum was decanted from the mass of blood cells, evaporated, and dried at about 100°. The mass of cells and fibrin still containing

TABLE I.

Analysis of Cow Blood.

	Dry serum.	Dry cells and fibrin.
	per cent	*per cent*
Copper	0.0044	0.0007
Iron	0.0140	0.1580
Manganese	Trace.	Trace.
Zinc	0.0096	0.010
Phosphorus	0.1420	0.0798
Calcium	0.1120	0.0256
Magnesium	0.145	0.309
Potassium	0.275	0.112
Sodium	3.53	1.151
Nitrogen	12.80	15.28
Total sulfur	1.544	0.579

TABLE II.

Analysis of Calf Liver.

	Moisture-free.
	per cent
Copper	0.0125
Iron	0.0358
Manganese	0.0003
Zinc	0.040
Phosphorus	1.1218
Calcium	0.028
Magnesium	0.161
Potassium	0.896
Sodium	0.772
Nitrogen	10.24
Protein	64.00
Total sulfur	0.584

some serum was dried in a similar manner. Each of the two portions was carefully ashed in silica dishes and analyzed with the results shown in Table I.

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In order to demonstrate whether or not the copper contained in red blood has any useful function, as its presence suggests, livers of young calves were obtained, dried, ground, and analyzed. With other portions of the livers the following experiment was performed with white albino rats. The analysis of the dry calf livers is given in Table II.

TABLE III.

Hemoglobin Content of Blood of Rats without and with Copper in Their Diet.

Lot 1.	Solution of ash of calf liver, copper removed.		Lot 2.	Solution of ash of calf liver.	
	Tallqvist method.	Fleischl method.		Tallqvist method.	Fleischl method.
Mar. 28, 1928.			Mar. 28, 1928.		
Rat 1	75	66	Rat 1	95	90
" 2	65	68	" 2	90	110
" 3	65	76	" 3	90	85
" 4	75	86	Average.....	91.7	95
Average.....	70	74	Apr. 3, 1928.		
Apr. 3, 1928.			Rat 1	95	87
Rat 1	68	70	" 2	100	104
" 2	68	80	" 3	90	85
" 3	68	83	" 4	90	90
" 4	70	78	" 5	78	87
" 5	75	77	Average.....	90.6	90.6
" 6	68	74	Apr. 4, 1928.		
" 7	75	86	Rats + 2 gm. dry calf liver per day.		
" 8	74	83	Rat 6	90	87
" 9	68	68	" 7	85	89
" 10	74	80	" 8	80	70
Average.....	70.8	77.9	Average.....	85	82

Two equal portions of the moisture-free liver were ashed separately in quartz dishes, the ash treated with hydrochloric acid, evaporated to dryness, taken up with a few drops of hydrochloric acid and a little water, and diluted with water to such a volume that 1 cc. contained approximately 0.1 mg. of iron. The HCl solution of the other portion of liver was treated with H_2S to precipitate the copper it contained, and the precipitate filtered out

and washed. The filtrate from the copper precipitate was evaporated to dryness with a few drops of nitric acid to oxidize ferrous iron to the ferric condition. Hydrochloric acid was added to the residue and evaporated to a pasty consistence and this operation was repeated twice, after which water was added to make the volume the same as the solution which contained copper.

Two lots of white albino rats, each rat weighing about 50 gm., were put into wire cages fitted with screen floors, two rats in each cage. The rats were fed skim milk in the morning and whole milk in the afternoon. The milk was placed in small glass tumblers attached inside the cages. Distilled water was also available to the rats, *ad libitum*. After the rats had been on the milk diet for about 4 weeks, 10 cc. of the solution without copper were added to the portion of skim milk given to one lot of rats once each day, and a like amount of the solution containing copper was added to the skim milk given to the second lot of rats once each day.

At the end of the 6th week, hemoglobin determinations were made from the blood of the two lots of rats, with the results shown in Table III.

The hemoglobin readings were made independently by Healy and McHargue, the average reading being taken. The analyses of the blood serum and cells and the liver were made by Hill.

When the samples of blood for the hemoglobin determinations were taken, it was observed that the blood from the rats from which copper was withheld was noticeably darker in color than the blood from the rats that received copper, the latter's blood being a brighter red. The samples of blood were obtained by clipping off sections of the tails of the rats, discarding the first few drops, and collecting the blood by means of a Fleischl blood pipette. Drops of fresh blood were absorbed on pieces of standard filter paper and immediately matched with the standard color chart in the Tallqvist method.

The rats that received copper in their diet possessed to a noticeable degree a pink ruddiness where the skin is exposed, for example on the tail, feet, and nose, which was absent on those from which copper was withheld.

From the external appearance of the rats, the color of the fresh blood, together with the hemoglobin readings, the authors infer

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that copper has an important function in the formation of hemoglobin and in the metabolism of animals having red blood.

Further experiments concerning the effect of copper, manganese, zinc, *etc.*, are under way.

Addendum.—Since this article was submitted for publication Hart, Steenbock, Waddell, and Elvehjem have published similar findings (11).

BIBLIOGRAPHY.

1. McHargue, J. S., The association of manganese with vitamins, *J. Agric. Research*, 1924, xxvii, 417.
2. McHargue, J. S., The occurrence of copper, manganese, zinc, nickel and cobalt in soils, plants and animals and their possible functions as vital factors, *J. Agric. Research*, 1925, xxx, 193.
3. McHargue, J. S., The association of copper with substances containing the fat-soluble A vitamin, *Am. J. Physiol.*, 1925, lxxii, 583.
4. McHargue, J. S., The significance of the occurrence of copper, manganese and zinc in forage crops and foods, *J. Am. Soc. Agron.*, 1925, xvii, 368.
5. McHargue, J. S., Manganese and plant growth, *Ind. and Eng. Chem.*, 1926, xviii, 172.
6. McHargue, J. S., Further evidence that small quantities of copper, manganese and zinc are factors in the metabolism of animals, *Am. J. Physiol.*, 1926, lxxvii, 245.
7. McHargue, J. S., Significance of the occurrence of manganese, copper, zinc, nickel and cobalt in Kentucky bluegrass, *Ind. and Eng. Chem.*, 1927, xix, 274.
8. McHargue, J. S., The proportion and significance of copper, iron, manganese and zinc in some mollusks and crustaceans, *Kentucky Acad. Sc.*, 1927, ii, 46.
9. McHargue, J. S., Metals in plants, their importance to humans, *Research Narratives*, 1927, vii.
10. Porter, J. A., Principles of chemistry, improved edition, New York and Chicago, 1875, 333.
11. Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, lxxvii, 797.

A MICRO METHOD FOR THE DETERMINATION OF POTASSIUM AS IODOPLATINATE.*

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(Received for publication, June 6, 1928.)

The standard and most widely accepted method for the determination of potassium depends upon its precipitation as potassium chloroplatinate. The modification called the Lindo-Gladding procedure (1) is official with the Association of Official Agricultural Chemists. Methods based upon the precipitation of potassium as potassium of cobalti-nitrite (2), have been very useful in determination of potassium in the blood and serum when the technique described (3) is rigidly adhered to, but under slightly different conditions, or for material of varying salt composition, are questionable. This criticism applies also to modifications involving either the gasometric (4) or colorimetric (5) determination. The cobalti-nitrite precipitate is not of constant composition (6).

The method about to be presented depends upon the precipitation of the potassium chloroplatinate in the ash of physiological material and its subsequent determination by colorimetric or titrimetric procedure or by both.

The quantitative determination depends upon the conversion of potassium chloroplatinate to potassium iodoplatinate by the addition of potassium iodide. Because of the deep rich wine color of the iodoplatinate in solution, amounts as small as 0.1 mg. of potassium in this form can be estimated by colorimetric comparison with known amounts of potassium chloroplatinate also treated in this way. The principle was used in 1903 by Cameron and Failyer (7) for the determination of potassium in water. The presence of iodine in the compound permits the use of the

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method of Peterson (8), in which potassium iodoplatinate is reduced in neutral solution to K_2PtI_4 with sodium thiosulfate.

The probable reactions taking place are expressed in the following equations.

$$K_2PtCl_6 + 6KI = K_2PtI_6 + 6KCl$$
$$K_2PtI_6 + 2Na_2S_2O_3 = K_2PtI_4 + 2NaI + Na_2S_4O_6$$
$$(Pt^{++++} + 2S^{++} = Pt^{++} + 2S^{+++})$$

The color varies in proportion to the amount of potassium chloroplatinate present when potassium iodide is added to solutions of this salt. This proportion is maintained from 0.2 mg. per cent up to 10.0 mg. per cent, which is the highest concentration tried. Dilution does not affect the proportion. The color given by 1.6 mg. per cent and more cannot be read accurately in the colorimeter without previous dilution.

The rate of color development is proportional to the amount of potassium iodide present. The presence of acid also hastens color development, but after a time excess acid causes the liberation of iodine. The presence of free iodine destroys the proportional variation of the color. Heat also increases the rate of color formation. By the color development in 0.4 N potassium iodide (5 cc. of 2 N KI in 25 cc.) in the presence of either acid or hot water, full color is developed immediately.

The color so formed is permanent for 24 hours or longer. The only interfering substances found are iron, which forms a colored iodide, copper, which precipitates out as the iodide in acid solution, ferricyanide, which gives a green color, and alcohol, which reduces the potassium iodoplatinate. The hotter the solution, the less actual color is obtained, but this color is still in proportion to the amount of potassium present. Exposure to bright sunlight through glass for 30 minutes liberates iodine, which obviously invalidates the determination. Indirect light or darkness does not cause oxidation of the iodine salt.

By application of the optimal conditions defined above to the production of the wine-red color, the amount of potassium present in a solution can be determined colorimetrically within the reading error of the instrument, which is considered as ± 4 per cent.

The potassium iodoplatinate is readily reduced in neutral solution by sodium thiosulfate. This reaction must take place

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in a solution exactly neutral, as acid frees excess iodine and alkali suppresses the reaction. A final concentration of normal potassium iodide and heat are both used to insure a rapid formation of the iodine compound. Heat does not affect the actual titration with thiosulfate. Alcohol naturally must be avoided. Neutral salts such as potassium chloride have no effect. In the preliminary work the precipitate was separated and washed by centrifugalization. This procedure gave good results, but an occasional analysis in a series gave large variation. This led to an improved filtration method which we have adopted here.

Because of the color of the reduced salt in solution, which is a lemon-yellow, the iodine salt is a self-indicator. The end-point is taken as the place at which 1 drop of the thiosulfate changes the color of the solution from a reddish to a greenish yellow.

By titration of the iodoplatinate solution with 0.01 N sodium thiosulfate in a micro burette, 0.4 mg. potassium or more can be determined with an accuracy of ± 2 per cent.

The reduced salt tends to become reoxidized on standing in the air, especially in acid solution. Advantage is taken of this fact to check the titration value with a colorimetric comparison after complete oxidation. Quantitative transformation to potassium iodoplatinate is hastened by the addition of acid and a mild oxidizing agent, 0.2 per cent H_2O_2.

Both the ashing of the sample and the precipitation of the potassium chloroplatinate are carried out according to the well known Lindo-Gladding method for potassium. A few minor modifications have been introduced to make the procedure applicable to such small amounts of material as must be used. With materials such as amino acids which contain large amounts of nitrogenous substances in comparison to the potassium content, a preliminary ashing in glass with sulfuric acid and superoxol is recommended. Otherwise, 5 to 10 per cent of the potassium is lost. When a small amount of protein is present, as in pathological cerebrospinal fluids, the results may be 50 per cent low unless the protein is first removed. The potassium chloroplatinate is precipitated in the presence of redistilled alcohol and the wash liquids are saturated with the salt. In this way the last 0.01 mg. is precipitated.

Ammonia also forms a chloroplatinate which has the same

solubilities and properties as potassium chloroplatinate. Because of this, great care should be taken that all ammonia is expelled from the ash. A sulfuric acid ashing should be carried out on all samples just before the precipitation of the chloroplatinate to insure the volatilization of absorbed ammonia.

Analytical Procedure.

1. Reagents.

Trichloroacetic acid, 20 per cent.

Superoxol (30 per cent hydrogen peroxide) Merck, Blue Label.

Sulfuric acid, approximately 4 N.

Hydrochloric acid, approximately N.

Chloroplatinic acid, 10 per cent platinum.

Alcohol, redistilled over lime.

Alcohol, redistilled over lime, saturated with potassium chloroplatinate.

Potassium chloride, 10 per cent, saturated with potassium chloroplatinate.

Potassium iodide, 2 N, iodate-free, recrystallized from alcohol.

Sodium thiosulfate, 0.01 N. This is standardized against potassium iodate.

Hydrogen peroxide, 0.2 per cent (made up just before use).

2. Preparation of the Sample.

(*a*) *Blood Serum or Plasma.*—The determination is made on the protein-free filtrate. 1 volume of 20 per cent trichloroacetic acid is added to 1 volume of serum or plasma and diluted to 5 volumes. 5 cc. of the filtrate, equivalent to 1 cc. of the serum or plasma, are taken for the colorimetric determination. Twice this amount is taken for the titrimetric determination.

(*b*) *Whole Blood.*—1 volume of whole citrated blood is precipitated with 2 volumes of 20 per cent trichloroacetic acid and diluted to 5 volumes. 1 cc. of the human blood filtrate, which is equivalent to 0.25 or 0.35 mg. of potassium, is taken for the colorimetric procedure and twice the amount for the titrimetric determination. 2 to 5 cc. of dog blood filtrate are necessary to supply the correct amount of potassium.

3. Ashing of Sample.

(*a*) *Salt Solutions.*—These are ashed directly.

(*b*) *Serum, Plasma, and Cerebrospinal Fluid.*—An amount of the sample containing from 0.15 to 0.80 mg. of potassium is

placed in a Folin digestion tube. 0.6 cc. of 4 N sulfuric acid is added and the mixture is evaporated to a small volume. A drop or more of superoxol (30 per cent hydrogen peroxide) is added at intervals and the ashing is continued until a clear, colorless solution is obtained while sulfuric acid fumes are escaping. Care should be taken that all hydrogen peroxide has been dispelled before one continues. The sample is then transferred to a platinum crucible, evaporated to a small volume, and ashed according to the regular procedure described in (*c*).

(*c*) *Whole Blood, Urine, and Stool.*—An amount of the sample containing from 0.15 to 0.8 mg. of potassium is transferred to a platinum crucible. 4 drops of 4 N sulfuric acid solution are added. The sample is evaporated to dryness on a hot plate. The dish is heated carefully over a micro burner until all sulfuric acid fumes are given off and the ash becomes white. 1 drop of 4 N sulfuric acid is added and the sample re-ashed at red heat. With samples containing a larger percentage of potassium than that in blood, such as stool, it is often easier to ash more of the sample than is required for one determination. This is dissolved in a definite volume of water and aliquots are taken for the determination.

4. Precipitation of Potassium Chloroplatinate.

To the ash so prepared are added 1 drop of N hydrochloric acid and 0.30 cc. of platinic acid (10 per cent platinum). This is thoroughly mixed and 5 cc. of alcohol are added. After standing for 20 minutes, the precipitate is transferred to a micro filter (9). The filter is made by mounting a 1 inch funnel in a Witt filtering apparatus, which is essentially a suction flash with a ground glass removable top, so that the filtrate may be recovered in a small inner tube. A glass pearl is dropped into the funnel and a mat about $\frac{1}{32}$ of an inch thick of fine grained asbestos is put over the bead. This makes essentially a micro Caldwell crucible. The excess platinum is filtered off by suction and saved for recovery. The precipitate and the filter are then washed four or five times with alcohol saturated with potassium chloroplatinate. The contaminating salts, which are precipitated with the chloroplatinate in alcohol, are washed out with 3 or 4 washings of 10 per cent potassium chloride saturated with potassium chloroplatinate.

5. Quantitative Determination of Potassium Chloroplatinate.

The amount of potassium in the chloroplatinate so obtained can be determined in the following ways.

(*a*) *Colorimetric Determination.*—A 25 cc. volumetric flask is placed in a Witt filtering apparatus and the precipitate dissolved *in situ* by the repeated addition of small amounts of hot water which is first used to rinse the platinum crucible. This is followed by addition of 5 cc. of 2 N potassium iodide and 1 cc. of 1 N HCl. The mixture is cooled and made to the volume. The deep wine-red color can be compared against known standards at once.

TABLE I.

Recovery of Potassium from Known Solutions.

Authors' method.	Amount present.	Error.	Remarks.
mg.	*mg.*	*per cent*	
0.284	0.288	−1.3	Colorimetric procedure (centrifuged). Artificial blood salts.
0.289		+0.2	
0.288		±0	
0.267		−7.2	
0.292		+1.5	
0.581	0.576	−0.8	Titrimetric procedure (filtered). Artificial blood salts.
0.579		+0.5	
0.579		+0.5	
0.586		+1.6	
0.586		+1.6	
0.398	0.400	−0.4	Titrimetric procedure. K_2SO_4.
0.405		+1.0	

Colorimetric determination is convenient for 0.1 mg. to 4 mg. of potassium in the form of potassium chloroplatinate.

(*b*) *Titrimetric Procedure.*—The funnel containing the precipitate is removed from the Witt filtering apparatus, inverted, and the precipitate together with the asbestos returned to the original crucible by means of a small glass rod inserted in the stem of the funnel. The sides of the funnel are washed with 1 to 2 cc. of hot water and 1 cc. of 2 N potassium iodide. The mixture is heated almost to boiling for 3 to 5 minutes. It is not necessary to remove the asbestos. The solution is then titrated hot with 0.01 N sodium thiosulfate delivered from a micro burette calibrated to 0.01 cc.

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TABLE II.

Potassium Determinations on Physiological Material.

Material.	Authors' method.	Gravimetric method.		Remarks.	Material.	Authors' method.	Gravimetric method.		Remarks.
	mg.	*mg. in aliquot*	*mg. per cent*			*mg.*	*mg. in aliquot*	*mg. per cent*	
Human blood.	0.365	0.365	182	Titrimetric procedure.	Cow blood —Continued.	0.464	0.447	223	Titrimetric.
	0.365					0.470			
						0.460			
	0.357					0.466			
	0.368					0.450			
	0.357					0 451			
	0.356					0.460			
	0.368					0.460			Colorimetric.
	0.364					0.460			
	0.375					0.436			
	0.375					0.458			
						0.441			
	0.375					0 436			
	0.372					0.448			
Cow blood.	0.169	0.179		Colorimetric.	Rat blood.	0.909	0.940		Titrimetric.
	0.182					0.930			
	0.174					0.930			
	0.178					0.941			
	0.176								
						0.465	0.470	235	Titrimetric.
	0.182	0.179		Colorimetric.		0.463			
	0.181				Pig blood.	0.512	0.501	250	Titrimetric.
	0.174					0.520			
	0.175					0.516			
	0.178					0.512			
	0.174								
	0.177				Dog blood.	0.217	0.223		Colorimetric.
						0.223			
	0.087	0.089		Colorimetric.		0.225			
	0.086								
	0.084					0.446	0.446		Titrimetric.
	0 085					0.450			
	0.086								

TABLE II—*Concluded.*

Material.	Authors' method.	Gravimetric method.		Remarks.	Material.	Authors' method.	Gravimetric method.		Remarks.
	mg.	*mg. in aliquot*	*mg. per cent*			*mg.*	*mg. in aliquot*	*mg. per cent*	
Dog blood —Continued.	0.425 0.444	0.446	22.3	Colorimetric following titrimetric above.	Urine ashings.	0.449 0.449 0.449 0.433 0.426	0.436		Titrimetric.
						0.411 0.404	0.407		Titrimetric.
Cow serum.	0.238 0.229 0.226 0.242	0.239		Colorimetric (centrifuged).		0.408 0.404 0.417 0.418 0.422			
	0.461 0.454	0.478	23.9	Titration (centrifuged).		0.745 0.760	0.768		Titrimetric.
Cow's milk.	0.662 0.640	0.650		Titrimetric.		0.762			

The end-point is a lemon-yellow color free from red. 1 cc. of thiosulfate is equivalent to 0.39 mg. of potassium. The titrimetric procedure is convenient for 0.4 to 1.0 mg. of potassium and potassium chloroplatinate.

(*c*) *Combination.*—The titrimetric result can be checked colorimetrically by reoxidation of the solution. 1 cc. of the normal hydrochloric acid solution and 0.10 cc. of a 0.2 per cent hydrogen peroxide solution are added to the mixture. This is allowed to stand exposed to the air from 30 minutes to 1 hour. The solution is made up to 50 cc. and compared against a suitable colorimetric standard.

The results obtained by this procedure are given in Tables I and II.

Determinations were made in triplicate or quadruplicate with good agreement. It is needless to say that known solutions of potassium sulfate were determined correctly; that a mixture of

salts of the same composition as the blood and salts of known purity gave theoretical values for potassium; and that potassium added to various unknowns was recovered. In addition, varying amounts of material yielded the same percentage analysis for potassium. All results with the present method were checked by gravimetric determination according to the Lindo-Gladding method. The results for blood, serum, milk, and urine were also checked by this method.

SUMMARY.

A procedure is outlined for the determination of 0.1 mg. or more of potassium. Potassium chloroplatinate is precipitated and converted to iodoplatinate. Quantitative determination is made either by colorimetric comparison with known standards, or by titration with sodium thiosulfate. By this method 0.1 mg. can be determined, ±4 per cent, and 0.4 mg., ±2 per cent.

BIBLIOGRAPHY.

1. Official and tentative methods of analysis of the Association of Official Agricultural Chemists, Washington, 1920, 12.
2. Tisdall, F. F., and Kramer, B., Methods for the direct quantitative determination of sodium, potassium, calcium, and magnesium in urine and stools, *J. Biol. Chem.*, 1921, xlviii, 1. Kramer, B., and Tisdall, F. F., The direct quantitative determination of sodium, potassium, calcium, and magnesium in small amounts of blood, *J. Biol. Chem*, 1921, xlviii, 223.
3. Kerr, S. E., Studies on the inorganic composition of blood. I. The effect of hemorrhage on the inorganic composition of serum and corpuscles, *J. Biol. Chem.*, 1926, lxvii, 689, (694).
4. Kramer, B., and Gittleman., I., The gasometric determination of potassium, *Proc. Soc. Exp. Biol. and Med.*, 1926, xxiv, 241.
5. Yoshimatsu, S., A colorimetric method for determination of potassium with 0.2 cc. of blood, *Tohoku J. Exp. Med.*, 1926–27, viii, 174.
6. Bowser, L. T., Some features of the cobalti-nitrite method for potassium, *U. S. Dept. Agric., Bureau Chemistry, Bull. 152;* 1912, 45.
7. Cameron, F. K., and Failyer, G. H., The determination of small amounts of potassium in aqueous solutions, *J. Am. Chem. Soc.*, 1903, xxv, 1063.
8. Peterson, H., Volumetrische Bestimmung des Goldes und Platins, *Z. anorg. Chem.*, 1899, xix, 59.
9. Shohl, A. T., Apparatus for micro-filtration, *J. Am. Chem. Soc.*, 1928, l, 417.

THE RESORPTION OF COPPER AND FERROCYANIDE IONS BY COAGULATED PROTEINS.*

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It has been known for a long time that proteins are precipitated by, and precipitate, copper and other heavy metals; likewise, many acids behave in a similar way toward soluble proteins. Harnack (1) believed he showed a definite composition of the copper egg albumin precipitate. Galeotti (2), on the other hand, thought he found that copper is distributed between the protein precipitate and the solution according to the phase rule. Many other workers have come to varying conclusions concerning the nature of the compounds of copper and other heavy metals with proteins. Mathews (3) states that ions form salts with proteins, but does recognize that copper and other heavy metals may enter into combination with the amino group of the protein molecule. Loeb (4) has shown that the combination between gelatin and hydroferrocyanic acid is of the nature of a salt.

In view of the recent attention which has been given to the presence of small amounts of certain elements in the animal organism, it has been of interest to study the concentration of copper and of hydroferrocyanic acid on coagulated protein from very dilute solutions.

Methods.

Coagulated protein was used in these experiments because this made possible the separation of the protein for analysis from the solution with as little manipulation as possible. It was necessary only to filter off the coagulated protein and weigh the fraction collected. Had uncoagulated protein been used, it would have been necessary to precipitate the protein by some method which probably would have disturbed the equilibrium between the

* A preliminary report of this work was read before the American Society of Biological Chemists at Ann Arbor, April 13, 1928.

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copper in solution and the copper which had combined with the protein.

Coagulated egg albumin and coagulated edestin were used in these experiments. In order to save space, this discussion will be confined to the experiments with egg albumin. The coagulated egg albumin was prepared from crystallized egg albumin. It was thoroughly washed to remove salts and other soluble substances, and was found to be entirely free from copper but contained a very faint trace of iron.

In every case 1 gm. of coagulated protein was used. In most cases the protein was suspended in 2810 cc. of solution which contained 10 mg. of copper or 10 mg. of iron in the form of potassium ferrocyanide. In a few experiments, the volume of the solution was 5000 cc. which contained 5 mg. of copper. 1 gm. of protein was used in each of these experiments. In certain cases, the volume of solution was very slightly increased by the addition of acid or alkali in adjusting the pH of the solution. In many of the experiments with copper, no attempt was made to regulate the hydrogen ion concentration of the solution, which doubtless accounts for the rather wide fluctuations in the results obtained. In some of the later experiments, the pH was regulated and varied from pH of 3.4 to 6.9. Some experiments were carried out at pH lower than 3.4, but since the protein took up no copper, they are not reported here in detail. No experiments were carried out at pH above 7. It was feared the copper might be precipitated as the hydroxide above this point.

In the experiments with potassium ferrocyanide, the pH was varied between 3 and 6.3. Experiments with ferrocyanide were not attempted at higher acidity than that represented by pH 3, because even at this level there is some evidence of the decomposition of ferrocyanide. The protein was allowed to remain in solution from 2 to 6 days, but all evidence seemed to indicate that equilibrium had been reached in 2 days.

In filtering the protein, no effort was made to collect all of the protein, but 75 to 90 per cent of it was collected on an ash-free filter paper and washed with a very little water, drained with the aid of reduced pressure, and dried to constant weight. From the determination of copper or iron in this portion of the protein, the amount in 1 gm. was calculated.

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The copper was determined by a method which has been previously reported by the writer (5), and the iron, at first, by the method of Elvehjem and Hart (6) and later by the method of Kennedy (7).

Results.

In order to study adequately the results of these experiments, it seems necessary to pay careful attention, not only to the actual amount of copper and of iron combined with 1 gm. of protein, but also to the ratio between the amount of copper or iron combined with 1 gm. of the coagulated protein and the amount found in an equal amount of the solution.

The results of the first series of experiments showed that the average ratio between copper in protein to that found in an equal amount of solution was 2974 for eight experiments. An average of 5.05 mg. of copper, or 50.0 per cent of the total copper present, was taken up by the protein. The maximum variation in this series of experiments was quite large, being 1.72 mg., or 17.2 per cent of the total.

The results obtained in this series of experiments raise the following questions. (1) Will coagulated protein take up only half of the copper from solutions under conditions such as were maintained in this series of experiments? (2) What are the factors which cause the quite large variations in the amount of copper taken up by protein? (3) Is this variation produced by variations in hydrogen ion concentration or is it the result of some surface relation between the protein and the solution?

In order to obtain an answer to the first of these questions, the volume of the solution was increased to 5010 cc. in two experiments. It was found that the actual amount of copper taken up was not much different from that in the experiment in which the volume was only 2810 cc. The ratio of concentration of copper in protein to that in the solution was markedly increased, being about 6000. These experiments indicate that dilution does not diminish to any extent the amount of copper which will be taken up by protein, providing the amount of copper in the system remains unchanged. Two other experiments were carried out in which the volume was 5005 cc. and only 5 mg. of copper were present. In these experiments, the actual amount of copper taken up was

only slightly less than in those experiments in which the volume was only 2800 cc. and the amount of copper was 10 mg. The per cent of the total copper taken up, however, was much greater, being 95.8 and 81.8. In one case the ratio between copper in the protein and the copper in the solution rose to the very high value of 114,319. This, it is believed, shows a very high affinity of coagulated protein for copper, at least until about 5 mg. of copper have

TABLE I.

1 gm. of coagulated egg albumin brought to equilibrium with 2800 cc. of distilled water, 10 cc. of a solution of copper sulfate which contained 10 mg. of copper, and enough hydrochloric acid or sodium bicarbonate to bring the solution to the desired pH.

Date.	pH	Cu in 1 gm. protein.	Cu in solution.	Total Cu in protein.	Cu in solution.
1927		*mg.*	*mg.*	*per cent*	*p. p. m.*
July 8–14	3.4	0.302	9.698	3.02	3.44
" 8–14	3.4	0.302	9.698	3.02	3.44
" 8–14	3.5	0.436	9.564	4.36	3.39
" 8–14	3.5	0.455	9.545	4.55	3.38
June 27–30	4.3	1.39	8.61	13.9	3.06
" 27–30	4.3	1.77	8.23	17.7	2.92
July 8–14	5.2	2.55	7.45	25.5	2.651
" 8–14	5.2	2.54	7.46	25.4	2.654
" 8–14	5.2	2.75	7.25	27.5	2.58
" 8–14	5.6	3.22	6.78	32.2	2.41
" 21–27	6.9	5.87	4.13	58.7	1.47
" 21–27	6.9	5.73	4.27	57.3	1.52
" 21–27	6.9	6.00	4.00	60.0	1.42

been taken up by each gm. of the protein. These experiments show that dilution does not influence the taking up of copper by protein in any important way, providing the total amount of copper in the mixture is not reduced below 5 mg.

In these more dilute solutions, there is considerable variation in the amount of copper taken up by the protein. The very small amount of copper left in solution makes the ratio very high and

also increases the variation in these ratios because experimental errors are multiplied by such large figures. It appears certain, however, that this fluctuation is not all due to experimental error.

The study of these fluctuations in the amount of copper taken up by coagulated protein dealt entirely with the effect of varying the

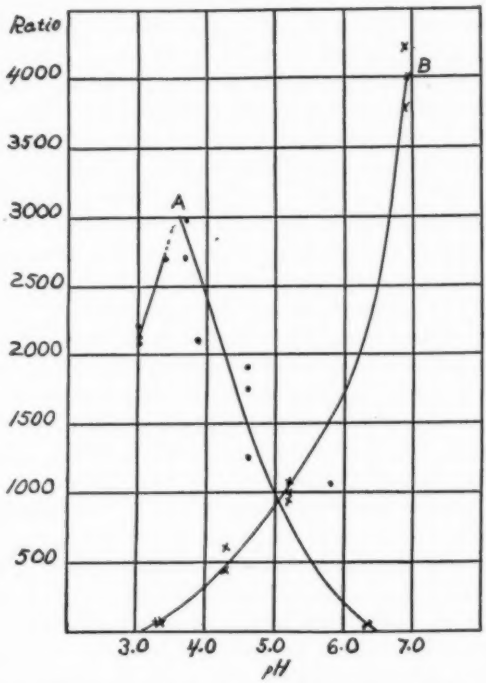

FIG. 1. Showing the effect of pH upon the distribution of ions between coagulated egg albumin and water. *A*, ferrocyanide; *B*, copper.

hydrogen ion concentration of the solution. In these experiments, 2800 cc. of water, 10 cc. of a solution containing 10 mg. of copper in the form of the sulfate, and enough of a solution of hydrochloric acid or sodium bicarbonate (see Table I for exact volume) to obtain the desired pH were allowed to come to equilibrium with 1 gm. doses of coagulated egg albumin. Solutions of pH from 2.5 to

6.9 were used in these studies. It was found that the protein took up no copper at a pH of 2.5 and virtually none at pH 3, so that Table I includes results only from pH 3.4 to 6.9. No experiments were carried out above pH 6.9 on account of the possible danger of the precipitation of copper at higher pH values. The ratio of copper combined with the protein to that in an equal amount of the solution varied from an average of 3995 at a pH of 6.9 to 87.5 at a pH of 3.4 and, of course, to 0 at a pH of 2.5. There was found in the protein an average of 58.3 per cent of the total copper when the pH was 6.9 and 3.02 per cent at a pH of 3.4. The variations between these extremes are shown in Table I and in Fig. 1. It will be noted that the curve in Fig. 1 resembles a titration curve to a certain extent. The number of determinations reported is not sufficient to enable one to say that the form of the curve is correct, but it is felt that it shows in an approximate manner the variation of the amount of copper which will combine with coagulated protein at varying hydrogen ion concentrations.

In view of this variation of the combination of copper with protein at different hydrogen ion concentrations, it seemed worth while to investigate the combination of a negative ion with coagulated egg albumin at varying hydrogen ion concentrations. As mentioned above, ferrocyanide was used for this purpose. The experiments with ferrocyanide were carried out exactly the same as in the preceding series of experiments, except that potassium ferrocyanide which contained 10 mg. of iron was used in place of copper. Table II and Fig. 1 give the variation in the combination of ferrocyanide with coagulated protein at different pH values. The results are calculated in mg. of iron combined with 1 gm. of protein and the ratio between the amount of iron in 1 gm. of protein to the iron in an equal amount of solution. It will be noticed that the protein contained an average of 51.04 per cent of the total iron, and the ratio was 2908 at a pH of 3.7, while at pH of 6.3 an average of only 1.72 per cent of all the iron was found in the protein, and the ratio was 45.7. There was a definite decrease in the amount of iron combined with the protein in solutions more acid than pH of 3.7. It is believed that this is to be accounted for by the decomposition of hydroferrocyanic acid in acid solution. In fact, the work with ferrocyanide was made unsatisfactory by this tendency of the ferrocyanide. These results, although very few

in number, do show that coagulated protein combines with ferrocyanide and presumably with other anions to a greater extent in more acid solutions.

The type of combination between the coagulated protein and the copper and ferrocyanide appears to be the same as with uncoagulated protein. If this combination is of the nature of

TABLE II.

1 gm. of coagulated egg albumin brought to equilibrium with 2800 cc. of distilled water, 10 cc. of a solution of potassium ferrocyanide which contained 10 mg. of iron, and enough hydrochloric acid or sodium bicarbonate to bring the solution to the desired pH.

Date.	pH	Fe in 1 gm. protein.	Fe in solution.	Total Fe in protein.	Fe in solution.
1927		*mg.*	*mg.*	*per cent*	*p. p. m.*
July 31–Aug. 4	3.0	4.28	5.72	42.8	2.03
" 31– " 4	3.0	4.38	5.62	43.8	1.99
" 31– " 4	3.0	4.31	5.69	43.1	2.01
" 31– " 4	3.0	4.24	5.76	42.4	2.04
" 31– " 4	3.4	4.84	5.16	48.4	1.832
" 31– " 4	3.4	4.865	5.135	48.65	1.823
Aug. 15–17	3.7	5.308	4.692	53.08	1.77
" 15–17	3.7	4.90	5.10	49.00	1.81
" 15–17	3.9	4.25	5.75	42.5	2.04
" 15–17	4.6	4.05	5.95	40.5	2.11
" 15–17	4.6	3.08	6.92	30.8	2.45
" 15–17	4.6	3.85	6.15	38.5	2.18
" 7–11	5.8	2.74	7.26	27.4	2.544
" 7–11	6.3	0.165	9.835	1.65	3.93
" 7–11	6.3	0.190	9.81	1.90	3.92

adsorption, it is surprising that the results are so constant at a definite pH, for it cannot be supposed that any two doses of dried coagulated egg albumin or edestin would present the same surfaces to the solutions. It appears, therefore, that the combination between coagulated protein and the copper and the ferrocyanide is a chemical one in the same sense that uncoagulated protein combines with acidic or basic radicals.

SUMMARY.

It is shown that coagulated proteins take up copper from dilute solutions, and if the solutions are dilute enough, almost all of the copper may be taken out of solution.

The amount of copper taken up by coagulated egg albumin increases with the pH at least up to the pH of 6.9.

Preliminary studies indicate that the coagulated protein takes up the acid radical ferrocyanide in acid solution and the amount of ferrocyanide combined increases as the pH decreases to the pH of 3.7.

BIBLIOGRAPHY.

1. Harnack, E., *Z. physiol. Chem.*, 1881, v, 198.
2. Galeotti, G., *Z. physiol. Chem.*, 1903, xl, 492.
3. Mathews, A. P., Physiological chemistry. A text-book and manual for students, New York, 4th edition, 1925.
4. Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 2nd edition, 1924.
5. Hendrix, B. M., *J. Biol. Chem.*, 1927, lxxiv, p. vii.
6. Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1926, lxvii, 43.
7. Kennedy, R. P., *J. Biol. Chem.*, 1927, lxxiv, 385.

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THE EFFECT OF SODIUM CITRATE, ACETATE, AND LACTATE ON THE ULTRAFILTRABILITY OF SERUM CALCIUM.

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The toxicity of sodium citrate was described by Metcherlich (1) in 1845, but little was known of its mode of action in the animal organism until the studies of Sabatini (2) and of Loeb (3) in 1901. Loeb (3) and Zoethout (4) attributed the tetanic contractions produced in muscles immersed in sodium citrate or oxalate solutions to the formation of insoluble calcium salts of citrate and oxalate. This explanation of the toxicity of sodium citrate has been questioned by Gros (5) who demonstated that sodium tartrate does not produce convulsions when injected intravenously, although calcium tartrate is less soluble than calcium citrate. Sabatini (6) found that the quantity of the serum calcium does not alter after sodium citrate injection, and he therefore suggested that sodium citrate invokes a physical or chemical change in the serum calcium so that it becomes no longer available as a regulator of muscular contraction and relaxation. He concludes that since calcium citrate is soluble, it must be that it forms an unionized compound.

With the exception of Gross (7), who injected dogs subcutaneously with dilute solutions of sodium citrate, all modern investigators agree that this salt is capable of producing tetanic muscular contractions when introduced in the living animal body (8–14).

The following investigation suggested itself after an accidental observation. In the course of some dietary experiments with young rabbits, sodium citrate was added to their milk rations to promote digestibility, as recommended by Hart (15). On several occasions rabbits were found dead in their cages and even necropsy did not reveal the cause of death. In one instance, however, a small rabbit was observed to be in a state of tonic and clonic convulsions soon after partaking of the milk diet. We

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suspected that the ingested sodium citrate found its way into the circulation and formed an undissociable calcium compound, thereby causing convulsions. Blood was obtained by cardiac puncture and the serum calcium was found to be within the normal limits for rabbits of that age and size, and apparently in agreement with the observation of Sabatini. Part of the serum was ultrafiltered in a collodion bag[1] at a pressure of 120 to 150 mm. of mercury. The calcium concentration of the filtrate was determined and compared with the ultrafiltrate of normal rabbit serum. It was found that, whereas only 45 to 57 per cent of the serum calcium of normal rabbits is ultrafiltrable, nearly 100 per cent of the serum calcium of the citrate-fed rabbit passed into the filtrate.

The following experiments were planned to confirm this observation.

I. Effect of Intravenous Injection of Sodium Citrate on Ultrafiltrability of Serum Calcium.

Preliminary to injection, blood was obtained from large adult rabbits by cardiac puncture. Then the desired solution was injected into the marginal ear vein of the animal and as soon as convulsions appeared blood was again obtained by cardiac puncture. The blood was collected in dry, clean centrifuge tubes, allowed to clot, centrifuged for about 15 minutes, and the clear serum separated. Part of the serum was used for ultrafiltration. The ultrafiltrate, as well as the undialyzed serum, was analyzed for calcium by the method of Kramer and Tisdall (16).

In the first experiments 1 cc. of a 30 per cent solution of sodium citrate was employed and before the needle was withdrawn from the vein, tonic and clonic convulsions occurred. Within a minute, the animals were in opisthotonos, and died of respiratory and circulatory failure. On rare occasions animals were revived by the application of amyl nitrite to their nostrils as suggested by Normet (9).

When sodium citrate was injected in more dilute solutions so

[1] The bags were made of 6 per cent du Pont parlodion dissolved in 25 cc. of absolute alcohol and 75 cc. of ether. About three coats of the solution were sufficient to make a bag which would withold the desired pressure and render the filtrate protein-free.

that the rate of injection was slower, a greater quantity of this salt was necessary to produce convulsions and the effect did not appear until 3 to 4 minutes after injection. The effect on the ultrafiltrability of serum calcium was, however, the same as when

TABLE I.

Effect of Sodium Citrate on Ultrafiltrability of Serum Calcium of Rabbits.

Date.	Rabbit No.	Amount injected.	Concentration.	Before injection.		Per cent ultrafiltrable.	After injection.		Per cent ultrafiltrable.	Remarks.
				Ca in serum.	Ca in ultrafiltrate.		Ca in serum.	Ca in ultrafiltrate.		
1926		*cc.*	*per cent*	*mg. per cent*	*mg. per cent*		*mg. per cent*	*mg. per cent*		
Feb. 1	1	2.0	30	14.6			15.2			Convulsions within 1 min.
" 1	2	1.0	30	16.8	7.3	45	14.3	12.0	86	" "
" 5	3	11.0	10	13.9	8.3	57	14.4	13.2	97	Convulsions within 3 min.

TABLE II.

Effect of Addition of Sodium Citrate to Serum in Vitro on Ultrafiltrability of Serum Calcium.

Source of serum.	Amount of serum.	Amount of 30 per cent citrate added.	Before addition.		Per cent ultrafiltrable.	After addition.		Per cent ultrafiltrable.
			Ca in serum.	Ca in ultrafiltrate.		Ca in serum.	Ca in ultrafiltrate.	
	cc.	*cc.*	*mg. per cent*	*mg. per cent*		*mg. per cent*	*mg. per cent*	
Sheep.	15.0	1.0	10.7	4.9	45	10.6	10.4	99
"	15.0	1.0	10.0	6.4	64	10.0	8.9	89
"	15.0	1.0	9.6	4.2	43	9.6	9.6	100
Human.	15.0	1.0	10.7	4.5	42	10.7	8.9	84
"	15.0	1.0	10.7	4.8	45	10.7	10.5	98
Cow.	10.0	0.5	10.4	5.0	48	10.4	10.2	99
"	10.0	0.5	10.4	5.0	48	10.4	10.0	98

a more concentrated solution was employed. Table I shows that whereas only 45 to 57 per cent of the serum calcium of normal rabbits is ultrafiltrable, the serum calcium of rabbits injected with sodium citrate is 86 to 97 per cent ultrafiltrable.

II. Effect of Sodium Citrate on Ultrafiltrability of Serum Calcium in Vitro.

That the effect of sodium citrate on the state of serum calcium is the same whether added *in vivo* or *in vitro* may be seen in Table II. It shows that similar results are obtained *in vitro* with the serum of sheep, cow, and man.

III. Effect of pH on Ultrafiltrability of Serum Calcium in Vitro.

Although the effect of sodium citrate on the diffusibility of calcium is the same as if acid were added to serum, as can be inferred from Table III, where the pH was adjusted to 5.1 with

TABLE III.

Effect of pH on Ultrafiltrability of Serum Calcium.

Source of serum.	Amount of serum.	Amount of 30 per cent sodium citrate added.	pH	Ca in serum.	Ca in ultrafiltrate.	Per cent ultrafiltrable.
	cc.	*cc.*		*mg. per cent*	*mg. per cent*	
Cow.	10.0	0.0	7.2	10.4	5.0	48
"	10.0	0.5	8.3	10.4	10.2	99
"	10.0	0.5	8.2	10.4	10.0	98
"	10.0	0.5	5.9*	10.4	10.5	100
"	10.0	0.5	5.1*	10.4	10.6	100
"	10.0	0.0	5.1*	10.4	10.5	100

* Serum rendered acid by addition of HCl.

HCl without the addition of sodium citrate, yet the citrate effect is not due to acid. The citrate effect is evident when the serum is made more acid, equal to, or more alkaline than blood serum. If the change in reaction were the controlling factor, a decrease rather than an increase in the ultrafiltrable calcium should be expected, since the addition of sodium citrate renders the solution more alkaline.

IV. Effects of Sodium Acetate and Sodium Lactate.

The effects of sodium acetate and lactate were studied *in vitro* and *in vivo*. At first 30 per cent solutions of these salts were used even in correspondingly larger amounts than of the sodium citrate

TABLE IV.

Effect of Injection of Sodium Citrate, Acetate, and Lactate into Rabbits.

Date.	Rabbit No.	Amount of 30 per cent solution injected.	Before injection.			After injection.			Remarks.
			Ca in serum.	Ca in ultrafiltrate.	Per cent ultrafiltrable.	Ca in serum.	Ca in ultrafiltrate.	Per cent ultrafiltrable.	
1926		cc.	mg. per cent	mg. per cent		mg. per cent	mg. per cent		
June 17	4	6.0 Na acetate.	14.2	7.8	55	14.0	7.8	55	No convulsions.
" 18	4	1.0 " citrate.				12.0	12.0	100	Convulsions in 1 min.
		Stoichiometric amounts.							
1927									
Aug. 11	5	10.0 Na citrate.	15.8	8.5	53	15.8	16.0	100	Convulsions in 1 min.
	6	10.0 " acetate.	14.0	6.8	48	15.5	8.1	52	No convulsions.
	7	10.0 " lactate.	14.5	6.6	45	14.5	9.9	61	" "
	8	2.0 " citrate.	14.0	10.0	70	12.8	13.0	100	Convulsions at once.
	9	2.0 " acetate.	15.6	6.7	43	14.6	7.0	48	No convulsions.
	10	5.0 " lactate.	15.6	7.8	50	15.6	10.5	67	" "

TABLE V.

Effect of Addition of 0.2 Cc. of Stoichiometric Solutions of Sodium Citrate, Acetate, and Lactate on Ultrafiltrability of Serum Calcium (Sheep).

Amount of serum.	Solution added.	Ca in serum.	Ca in ultrafiltrate.	Per cent ultrafiltrable.
cc.		mg. per cent	mg. per cent	
15	None.	11.6	4.6	40
15	Na citrate.	11.6	9.9	85
15	" acetate.	11.6	4.9	42
15	" "	11.6	4.9	42
15	" lactate.	11.6	5.8	50
15	" "	11.6	5.7	49
15	" "	11.6	5.9	51

but no convulsions resulted nor was the ultrafiltrability increased to the extent obtained with sodium citrate. Later these experiments were repeated with stoichiometric concentrations of the three salts with similar results.

It is evident from Tables IV and V that, whereas sodium citrate evoked convulsions within 1 minute after injection, no convulsions occurred when sodium acetate or lactate was introduced; and that whereas the serum calcium is nearly 100 per cent diffusible when sodium citrate is present, the diffusibility is only slightly increased by the introduction of sodium lactate and not at all increased by sodium acetate.

DISCUSSION.

In 1911, Rona and Takahashi (17), employing the method of compensation dialysis, found that serum calcium exists in a diffusible and a non-diffusible form. The latter fraction, they suggested, was most likely bound to serum protein. Using the method of ultrafiltration, Cushny (18), Neuhausen and Pincus (19), von Meysenbug, Pappenheimer, Zucker, and Murray (20), Moritz (21), and Updegraff, Greenberg, and Clark (22) found that 45 to 50 per cent of the serum calcium is non-diffusible. Cameron and Moorhouse (23) proposed that the non-diffusible calcium is bound to some organic substance which may be the parathyroid hormone or is regulated by it. Salvesen and Linder (24) in their studies on nephritis noticed the parallelism between the reduction in serum calcium and protein. They also suggested that the non-diffusible calcium of the serum exists in a protein-like combination. Their indirect evidence has been elaborated by Loeb (25) and Marrack and Thacker (26). The former ascribes the diffusible and non-diffusible calcium to the "Donnan effect" and to a lesser extent to the formation of a complex calcium-protein compound. The latter investigators minimize the rôle of the "Donnan equilibrium" and explain their results mainly on the calcium-protein theory.

It is not the purpose of this paper to enter into a discussion regarding the state of the non-diffusible fraction of the serum calcium. It is desirous, however, to point out that our results in respect to the diffusible and non-diffusible portions of normal serum calcium agree well with those reported in the literature;

and in addition, to indicate the specific effect of sodium citrate on the non-diffusible fraction whether introduced *in vivo* or *in vitro*.

The effect of citrates on biologic systems has been known for nearly a century, but more recently their effect on systems containing calcium has been studied more systematically. Hastings, Murray, and Sendroy (27) demonstrated changes in the solubility of calcium phosphate and carbonate when sodium citrate was present; and Shipley, Kramer, and Howland (28) were unable to obtain calcification *in vitro* even with a Ca × P product of 60 if the calcium was present as calcium citrate.

As to the mechanism of the citrate effect the hypothesis of Sabatini that citrate ions combine with calcium to form a soluble, unionizable compound seems plausible. That calcium, although still soluble, is removed from solution when citrate ions are added was demonstrated by Shear and Kramer (29) in this laboratory. They studied the ionic interaction between calcium and citrate by means of conductivity titrations and found that the addition of a solution of sodium citrate to one of calcium chloride resulted in a binding of the calcium ions by the citrate. In subsequent experiments, performed at 38° Kramer, Shear, and Resnikoff (30) found that lactate and acetate behave normally towards calcium ions, *i.e.*, in the same manner as chlorides, while citrate ions show the effect noted above, *i.e.*, a diminished ionic activity as indicated by a decrease in conductivity.

Sabatini explained the convulsive seizures as due to the inactivation of calcium which is necessary for muscular contraction and relaxation. Normet attributes the shock phenomenon to the exciting action of sodium citrate on the sympathetic nervous system. Citric acid is absorbed by positive ions with the liberation of sodium, which is thus ready to take violently, from their places, the metallic ions of the tissues. This explanation of the mode of action of sodium citrate is supported neither by experimental evidence nor by theoretical speculation. Our ultrafiltration experiments and the observations of Shear and Kramer would seem to support Sabatini's hypothesis; namely, that the citrate ions combine with calcium to form an unionized compound and thus rob the organism of its active calcium necessary for muscular and nervous stimulation and inhibition.

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The beneficial effects of sodium citrate in peripheral hemorrhages have been attributed, by Neuhof and Hirshfeld (31), to a decrease in clotting time. This, however, has been questioned by Joannides (11). It is more plausible to explain the decrease in bleeding time as due to vasoconstriction as suggested by Goia and Petri (32) and by Normet, not because of the liberation of sodium ions but rather to the inactivation of calcium by citrate ions and thereby causing contraction of the muscular coats of the blood vessels. This seems plausible because of the resuscitating effects of vasodilators such as amyl nitrite in animals with sodium citrate convulsions.

From a therapeutic standpoint, it seems that dilute solutions of sodium citrate when injected intravenously cause vasoconstriction and prevent bleeding; while concentrated solutions cause convulsions and death. Joannides reports shock in a patient treated with intravenous sodium citrate, who subsequently recovered by the administration of calcium chloride; and Kionka (33) reports an immediate death in a patient so treated.

SUMMARY AND CONCLUSIONS.

1. Sodium citrate when injected intravenously into rabbits in 30 per cent solution produces almost instantaneous convulsions and death.

2. The serum calcium of such rabbits becomes nearly 100 per cent ultrafiltrable as compared to serum of normal rabbits which is only 45 to 47 per cent ultrafiltrable.

3. Sodium citrate when added to serum *in vitro* renders the calcium nearly 100 per cent ultrafiltrable.

4. The injection of sodium lactate or acetate into rabbits does not produce convulsions, nor do these salts increase the ultrafiltrability of serum calcium *in vitro* or *in vivo*.

5. These effects may be due to the formation of a soluble, unionizable calcium citrate compound.

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BIBLIOGRAPHY.

1. Metcherlich, C. G., Dissertation, Berlin, 1845, quoted by Salant (12).
2. Sabatini, L., *Arch. ital. biol.*, 1901, xxxvi, 417.
3. Loeb, J., *Am. J. Physiol.*, 1901, v, 362.
4. Zoethout, W. D., *Am. J. Physiol.*, 1902, vii, 199, 320.
5. Gros, O., *Arch. Exp. Path. u. Pharmakol.*, 1913, lxxi, 395.
6. Sabatini, L., *J. physiol. int.*, 1901, xxx, 2.
7. Gross, E. G., *J. Biol. Chem.*, 1923, lv, 729.
8. Gates, F. L., and Meltzer, S. J., *J. Exp. Med.*, 1916, xxiii, 655.
9. Normet, R., *Bull. Acad. méd.*, 1924, xcii, 1306.
10. Panisset, L., and Verge, I., *Comp. rend. Soc. biol.*, 1922, lxxxvii, 224.
11. Joannides, M., *Arch. Int. Med.*, 1926, xxxvii, 248.
12. Salant, W., and Wise, L. E., *J. Biol. Chem.*, 1916–17, xxviii, 27.
13. Salant, W., and Swanson, A. M., *J. Pharmacol and Exp. Therap.*, 1918, xi, 133.
14. Salant, W., and Hecht, S., *Am. J. Physiol.*, 1915, xxxvi, 126.
15. Hart, E. B., Steenbock, H., Elvehjem, C. A., and Waddell, J., *J. Biol. Chem.*, 1925, lxv, 67.
16. Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, xlviii, 223.
17. Rona, P., and Takahashi, D., *Biochem. Z.*, 1911, xxxi, 336.
18. Cushny, A. R., *J. Physiol.*, 1919–20, liii, 391.
19. Neuhausen, B. S., and Pincus, J. B., *J. Biol. Chem.*, 1923, lvii, 99.
20. von Meysenbug, L., Pappenheimer, A. M., Zucker, T. F., and Murray, M. F., *J. Biol. Chem.*, 1921, xlvii, 529.
21. Moritz, A.R., *J. Biol. Chem.*, 1925, lxiv, 81.
22. Updegraff, H., Greenberg, D. M., and Clark, G. W., *J. Biol. Chem.*, 1926–27, lxxi, 87.
23. Cameron, A. T., and Moorhouse, V. H. K., *J. Biol. Chem.*, 1925, lxiii, 687.
24. Salvesen, H. A., and Linder, G. C., *J. Biol. Chem.*, 1923–24, lviii, 635.
25. Loeb, R. F., *J. Gen. Physiol.*, 1925, viii, 451.
26. Marrack, J., and Thacker, G., *Biochem. J.*, 1920, xx, 580.
27. Hastings, A. B., Murray, C. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1926–27, lxxi, 723.
28. Shipley, P. G., Kramer, B., and Howland, J., *Biochem. J.*, 1926, xx, 379.
29. Shear, M. J., and Kramer, B., *Proc. Soc. Exp. Biol. and Med.*, 1927, xxiv, 624; *J. Biol. Chem.*, 1928, in press.
30. Kramer, B., Shear, M. J., and Resnikoff, L., unpublished data.
31. Neuhof, H., and Hirshfeld, S., *Ann. Surg.*, 1922, lxxvi, 1.
32. Goia, I., and Petri, I., *Paris méd.*, 1927, lxiii, 137.
33. Kionka H., quoted by Kobert, R., in Lehrbuch der Intoxikationen, Stuttgart, 2nd edition, 1896, i.

FACTORS AFFECTING THE ACCURACY OF THE QUANTITATIVE DETERMINATION OF VITAMIN A.*

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The quantitative determination of vitamin A by means of the rat growth method has in recent years become a matter of such importance as to repay extended study of the several factors influencing the accuracy of the results obtainable by the method as now developed.

The purpose of the present paper is to summarize, as concisely as possible, the findings of a number of experiments planned with special reference to this problem together with the results of a critical examination of the data of some hundreds of determinations of vitamin A which have been made in this laboratory in the course of investigations upon the distribution of this substance in nature and its behavior under various conditions. Except as otherwise explained, the general procedure in the experiments from which the data here discussed were drawn, has been that described by Sherman and Munsell.[1]

Selection of Experimental Animals.

All of the experiments here considered were made with young albino rats, 28 to 29 days old at the beginning of the "depletion period," *i.e.* when placed upon the vitamin A-free basal diet for depletion of the bodily store of this vitamin; and all were drawn from families which have been for many generations under observation in this laboratory and are of strictly known nutritional

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[1] Sherman, H. C., and Munsell, H. E., *J. Am. Chem. Soc.*, 1925, xlvii, 1639.

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history. As most of these families are being used in nutrition experiments continued through successive generations, our practise has been not to "breed from the best" but to use, as breeders, animals which are strictly representative of the average. Hence our animals are, at present, not so large as those of many laboratories. Another influence probably acting in the same direction is the fact that, in the experiments here described, we did not artificially reduce the number of young in any litter, nor restrict the number of times of breeding, but allowed each female to produce and rear as many young as she could. Under these conditions, most of our young rats weigh between 35 and 55 gm. when 28 days old, and in general only animals within these limits of size are used in experiments such as those with which the present paper deals.

Considerable numbers of experiments have been made with young from each of the two diets hitherto most largely used in this laboratory, *viz.* Diet A (Laboratory No. 16) and Diet B (Laboratory No. 13). Diet A consists of one-sixth dried whole milk and five-sixths ground whole wheat; Diet B, of one-third dried whole milk and two-thirds ground whole wheat. In each case sodium chloride is added in the proportion of 2 per cent of the weight of the wheat in the food mixture, and distilled water is furnished *ad libitum*. The young from families on the diet containing the larger proportion of milk are found to have larger bodily stores of vitamin A at a given age, as indicated by the fact that they require a longer time (and make more growth) upon the vitamin A-free diet before the bodily store of this vitamin is sufficiently depleted (or "diluted" by the growth of the animal) for gain in weight to cease. It has also been found that when young from Diets A and B are depleted of their surplus vitamin A in the same manner, those from Diet B still show higher vigor and greater resistance to common infections throughout the subsequent experimental period. Thus among the animals used in one series of experiments it was found that 75 per cent of those from Diet A, and only 25 per cent of those from Diet B, showed visible signs of infection on autopsy at the end of the experimental period. We therefore consider it more satisfactory to use young from families on the diet containing the higher proportion of milk even though these require a slightly longer depletion period.

In chemical terms the higher proportion of milk means, among other things, a diet richer in calcium, in certain of the amino acids, and in vitamin G (the more thermostable component of the vitamin B complex), as well as in vitamin A. It is hoped that the separate significances of these several factors may be shown in subsequent papers. Meantime it may be said that the experience of this laboratory seems to indicate that the vitamin A content of the family diet influences the vitality of the young, not only in respect to their bodily stores of this substance, but in some other way or ways as well.

Basal Diet.

The basal vitamin A-free diet, to which the young rats to be used for these experiments are transferred when 28 or 29 days old, consists of: purified casein, 18 per cent; dried yeast, 10 per cent; Osborne-Mendel[2] salt mixture, 4 per cent; sodium chloride, 1 per cent; corn-starch, 67 per cent (diet, Laboratory No. 379 a).

Sources of Vitamin D.

The antirachitic or vitamin D requirement was met (1) by irradiating the animals with a mercury vapor quartz lamp, (2) by irradiating the corn-starch entering into the basal diet, or (3) by adding to the basal diet 0.05 per cent of irradiated commercial cholesterol; as well as by the bodily store of vitamin D which the animals contained when separated from the mother and the family dietary at 28 days of age, and which *under our conditions* does not become depleted until long after the depletion of vitamin A if the young animals are kept upon a diet supplying neither vitamin A nor D.

Of the three methods just mentioned for supplementing the bodily store and insuring an abundant supply of vitamin D throughout the experiment for the determination of vitamin A, our experience inclines us to favor the feeding of irradiated commercial cholesterol or other form of irradiated ergosterol with, or as a supplement to, the basal diet; but it cannot be said that either of the other methods used was in any way consistently detrimental in our experience.

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1919, xxxvii, 572.

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Depletion and Test Periods.

Young rats from our colony, selected and fed as above described, usually gain about 35 to 50 gm. in body weight before growth ceases upon the basal diet; *i.e.*, during the depletion period. When growth has definitely ceased under conditions which extended experience indicates to mean the depletion of the bodily store of vitamin A, the animal usually also showing incipient signs of vitamin A deficiency, the depletion period is considered complete; each animal is placed in a separate all metal cage with raised screen floor to prevent access to excreta, and the test period or experimental period proper is begun.

Usually, of each litter of animals used in work of this kind, one or more is continued on the basal diet without supplement as a negative control which is observed daily for symptoms of the characteristic pathology of vitamin A deficiency. The experience thus gained ultimately enables the worker to detect these symptoms in their incipiency and thus to begin the test period a few days after growth has ceased on the basal diet and about 1 day before the appearance of distinctly sore eyes, the object being to have the animals thoroughly depleted of surplus vitamin A but not in a seriously pathological condition when the experimental or test period is begun. From this point onward, different test animals are fed different amounts of the food under investigation to determine what daily allowance of the food will furnish that amount of vitamin A just sufficient to permit the rat to grow at an average rate of 3 gm. per week; while the behavior of the negative controls gives assurance of the representative character of the animals which are being employed.

The nutritional requirement of the standard test animal thus becomes the basis for the measurement of the vitamin values of the foods or other materials tested; and, in the interest of accuracy of such determinations of vitamin A, we have studied the records of large numbers of our experiments to ascertain the extent to which the gains in weight during the test period are influenced by the sex of the experimental animal, or its size at the beginning of the test period.

Relation of Size of Experimental Animal.

The relation of the size of the experimental animal at the beginning of the test period to its gain in weight during that period

has been investigated by means of a critical study of the data of experiments in which two or three animals of the same sex but different size had been fed the same amount of vitamin A; and, in the cases here considered, always such an amount as to induce a gain at somewhere near the "unit" rate of 3 gm. per week. The data were grouped according to whether the animal at the end of the depletion and beginning of the test period was small (under 75 gm.), medium-sized (75 to 100 gm.), or large (over 100 gm.). In 81 cases it was possible to compare the results obtained upon a medium-sized animal with those of a small animal of the same sex; in twenty-eight cases medium-sized and large animals could be similarly compared; and in thirteen cases we had sets of three strictly comparable animals, one small, one medium-sized, and one large. Thus grouped, the data when averaged yielded the comparisons shown in Table I.

TABLE I.

Comparison of Gains of Small, Medium-Sized, and Large Test Animals When Fed the Same Amounts of Vitamin A (about Sufficient to Support a Unit Rate of Gain during Test Period).

Size of rat.	No. of cases.	Average weight at beginning of test period.	Average gain in 8 wks. test period.	Differences and their probable errors.
		gm.	*gm.*	*gm.*
Small.	81	65	26 ± 0.6	6 ± 0.9
Medium.	81	87	20 ± 0.7	
Medium.	28	89	25 ± 1.1	
Large.	28	115	15 ± 1.9	10 ± 2.2
Small.	13	69	30 ± 1.9	8 ± 2.5
Medium.	13	89	22 ± 1.7	
Large.	13	113	14 ± 1.6	8 ± 2.3

The figures in the last column of Table I show the extent to which, in each of the three groupings, the average gain (for 8 weeks) of the large or small animals differed from that of the directly comparable animals of medium size, together with the probable error of this difference. The differences are from 3 to 7 times as large as their probable errors, and are undoubtedly significant.

Hence it appears that in order to obtain results of the highest accuracy in quantitative determinations of vitamin A by the rat

growth method, only such animals as do not differ greatly among themselves in size at the end of the depletion period should be used. For conditions such as those of our laboratory, a minimum limit of 70 or 75 gm. and a maximum limit of 100 gm. at the end of the depletion period would seem to be desirable.

There is clearly a tendency toward an inverse relationship between the body weight of the animal and its rate of gain upon a given limited allowance of vitamin A. Undoubtedly, too, this may also be stated conversely; *i.e.*, that among animals of the same species and age the amount of vitamin A required to support a given rate of gain tends to vary with the size of the individual. This corresponds with the similar relationships previously established in the case of vitamin B.[3,4]

Comparison of Males and Females as Experimental Animals.

The earlier experience of this laboratory,[1] which indicated that males and females may be used interchangeably in determinations of vitamin A by the method here employed, with gains of about 3 gm. per week in the test period, has now been confirmed by the data of large numbers of experiments made in such manner as to permit of direct comparison of the quantitative results yielded by the two sexes.

Thus the average gain shown during the 8 weeks test period by 148 males was 24.6 gm., and by 148 directly comparable females was 22.3 gm.

Statistically, the average difference of 2.3 gm. (or slightly less than 0.3 gm. per week) is probably, though not certainly, significant. Practically, it seems so small as to be safely negligible, although for investigations in which an extraordinary degree of refinement is sought it might be slightly preferable to work entirely with animals of the same sex.

Probably any possible slight advantage theoretically attributable to discrimination between the sexes will actually, in experiments of this kind, be outweighed by the advantages of being able to make greater use of litter mate controls when both sexes are employed.

[3] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1922, liv, 739.
[4] Sherman, H. C., and MacArthur, E. H., *J. Biol. Chem.*, 1927, lxxiv, 107.

The very slight tendency toward more rapid growth on the part of males than of females of the same age and nearly the same size, will in practise tend to be offset by the larger size which the males will usually have attained at the beginning of the test period, when the animals will usually be about 2 months old. This equalizing influence can readily be further availed of, at the cost of rejection of very few animals, by specifying a slightly larger standard size for male than for female rats at the end of the depletion and beginning of the test period. Any laboratory might, for example, decide that only females between 70 and 95 gm. or males between 75 and 100 gm. should be used interchangeably as standard test animals for quantitative determinations of vitamin A by the rat growth method.

It should, however, be carefully noted that all the foregoing discussion of the relation of sex to rate of gain, refers to experiments in which the level of feeding of the vitamin A-containing food or other material to be tested is such as to induce about unit growth, *i.e.* gain in weight of about 3 gm. per week during the test period. Much larger differences are to be expected if the level of vitamin A feeding is considerably higher, for in this case something approaching a normal rate of growth may become possible, and, as is well known, the normal growth curve of the male rat is distinctly steeper than that of the female at this age. Thus when we fed about twice the amount of vitamin A-containing food required for unit growth, the average gain during the 8 weeks test period was: for 117 males, 57 gm.; for 117 females, receiving the same amount of vitamin A, 42 gm.

Length of Experimental Period.

The practise of this laboratory, and, we judge, of a large proportion of research workers elsewhere, has been to continue the test period for 8 weeks. Laboratories devoted to control work upon cod liver oil commonly use a test period of 5 weeks and this was, therefore, incorporated in the United States Pharmacopoeia method of determining vitamin A values. With both practises in use, and resulting data recorded in terms of the same unit, it becomes important to know whether this difference in length of test period will influence either the uniformity of the results obtained or their average numerical values.

Averaging the results of 104 of our experiments in which the test animals showed about unit growth, we find at the end of the usual 8 weeks test period a gain of 25.89 gm. (or 3.24 gm. per week) with a coefficient of variation of 26 per cent. These same experiments averaged at the end of the 5th week of the test period show a gain of 18.12 gm. (or 3.62 gm. per week) with a coefficient of variation of 45 per cent. Thus the shortening of the test period to 5 weeks would have resulted in findings somewhat more variable and tending to be higher. The average difference of 0.38 gm. per week in rate of gain is about one-sixth of that which results from doubling the vitamin intake under these experimental conditions; so that the shortening of the test period from 8 weeks to 5 weeks might be expected, as an average tendency, to increase by about one-sixth the numerical ratings of vitamin A values of materials thus tested.

Desirable Rate of Growth during Test Period.

The English investigators to whom we are indebted for the fundamentals of this method of determining vitamin A recommended that the least practicable gain in weight during the test period be taken as the basis for quantitative comparison.[5] After somewhat extended experience in this laboratory we adopted a rate of gain of 3 gm. per week during the test period as a satisfactory standard or unit rate; and this same unit rate has also been adopted by the United States Pharmacopoeia. A lower level of vitamin A intake tends to result in greater mortality or more frequent cases of serious illness among the experimental animals; and at higher levels of intake the rates of gain are not increased in full arithmetical proportion, as was plainly shown by data of Sherman and Munsell,[1] so that quantitative comparisons at higher rates of gain necessarily make the method less delicate.

Others have more recently suggested[6] that the adoption of a higher level of vitamin intake and rate of gain during the test period might make the method more satisfactory by inducing a more vigorous condition in the test animals. While undoubtedly the higher level of vitamin A intake does result in more

[5] Drummond, J. C., and Coward, K. H., *Biochem. J.*, 1920, xiv, 661.

[6] Dutcher, R. A., Honeywell, H. E., and Dahle, C. D., *J. Biol. Chem.*, 1927, lxxv, 85.

vigorous animals, our experience has not, on the whole, confirmed the view that the method is thereby made more accurately quantitative. As already noted, there is unavoidably a loss in delicacy of response to small increments of vitamin intake as the level of vitamin feeding is raised. We also find, as noted above, that the higher rates of growth, induced by higher levels of experimental feeding of the vitamin-containing material under investigation, are attended with less regular results as between male and female test animals. For young rats from our colony subjected to the régime already outlined in this paper, a level of vitamin A feeding which induces a gain of 3 gm. per week during the test period seems adequate for the maintenance of a sufficient degree of health for the purpose of these experiments; and it permits of more delicate testing than does comparison at a higher level because the higher the level of vitamin intake the less is the response induced by a given small increment in this intake. It was still conceivable that the higher level of vitamin feeding and consequent rate of growth might, through inducing a higher degree of health, result in more uniform findings if the experiments are so controlled as to prevent the introduction of variations due to the differing responses of the sexes at the higher levels. When this was done in 104 cases directly comparable with the like number of cases mentioned in the preceding section of this paper, our experiments showed coefficients of variability of 32 and 30 per cent at the ends of the 5th and 8th weeks, respectively, when the level of vitamin intake during the test period was twice that of the experiments above mentioned. Here the variability is found to be slightly (perhaps not significantly) greater at the higher level when the tests are continued for 8 weeks, while at this higher rate of growth there was less increase of variability when the test period was shortened to 5 weeks. On the whole, we find no certain gain in uniformity to balance the undoubted loss in delicacy when the higher rate of growth is employed as a standard by means of which to make quantitative comparisons of vitamin A values.

SUMMARY.

In experiments upon the quantitative determination of vitamin A by the rat growth method, the diet fed to the mother, and available also to the young until separated from her at the age of 4

weeks, was found to influence markedly the vitality of the young as shown by susceptibility to infection during the test period, even though variations in store of vitamin A in the body were eliminated by means of a preliminary depletion period.

Other conditions being the same, the larger the experimental animal at the beginning of the test period the less is its rate of gain upon a given limited intake of vitamin A. Doubtless this may with equal accuracy be stated in the converse form, that is, among animals of the same species and age, the amount of vitamin A required to support a given rate of gain in weight tends to vary with the size of the individual.

Under the conditions described, with a rate of gain of about 3 gm. per week during the test period, male and female rats may be used interchangeably as test animals.

In experiments in which the rate of gain was approximately the standard unit rate of 3 gm. per week, it was found that to have shortened the test period from 8 weeks to 5 weeks would have resulted in slightly more variable findings and a higher average rate of gain per week. The shorter test period therefore tends to give the material tested a higher numerical rating in vitamin A value.

The suggestion that a considerably higher rate of gain during the test period, such as about 6 gm. per week, be adopted as the basis for quantitative work in the determination of vitamin A by the rat growth method, is studied by examination of the data of over 100 experiments at each of the two levels in question. While attractive in some respects, this suggestion, of a change to a standard rate of gain distinctly higher than the present unit rate of 3 gm. per week during the test period, is not, on the whole, found desirable, because it decreases the delicacy of the method without apparently increasing the accuracy. The present investigation therefore supports the continued use of the present unit rate of 3 gm. per week of gain in body weight in a standard test animal depleted of surplus bodily store of vitamin A, as the basis for quantitative work in the determination of vitamin A by the rat growth method.

THE COPPER CONTENT OF MILK.

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The presence of copper in cow's milk has been reported by several investigators (1–5). The most extensive investigation was that of Supplee and Bellis (2) who determined the copper content of freshly drawn milk of a large number of cows fed on different rations. They found that the copper content per liter varied from 0.2 to 0.8 mg., with no apparent correlation between copper content and ration. The variation in results obtained by investigators in widely different localities suggests the possibility of the influence of the copper content of native feed products. The analyses in this laboratory of milk collected from four states would indicate, however, that we can expect quite constant copper content of raw cow's milk. The copper content of milk of the sheep and of the goat was found to be about the same as that for the cow. The copper content of goat milk was low in all cases but always easily detectable notwithstanding the report to the contrary by Titze and Wedemann (6).

EXPERIMENTAL.

Raw Cow's Milk.—The samples of milk were collected in glass containers, directly from the cows. The possibility of contamination by copper during the analysis was always checked by means of equivalent quantities of copper-free water which were treated in the same manner as the milk. The copper was determined by the xanthate colorimetric method as modified by Supplee and Bellis (2). Each determination in Table I represents the average of three determinations of 100 cc. each of the milk from a given cow. Apparently the possible variation in copper content of native feeds, such as the grass in Kentucky (7), has little or no effect.

Raw Sheep Milk.—Samples of milk from a local herd gave the results recorded in Table II. The copper content is in very good agreement with that of cow's milk.

TABLE I.

Copper Content of Raw Whole Cow's Milk.

Herd by locality.	Animal No.	Copper.
		mg. per l.
Iowa.		
Mt. Vernon.	1	0.43
	2	0.42
	3	0.38
Cedar Rapids.	1	0.42
	2	0.45
	3	0.49
Luzerne.	1	0.49
	2	0.52
South Dakota.		
Manfield.	1	0.43
	2	0.42
Montana.		
Willard.	1	0.28
	2	0.26
Kentucky.		
Catlettsburg.	1	0.37
	2	0.40

TABLE II.

Copper Content of Raw Whole Sheep Milk.

Animal No.	Copper.
	mg. per l.
1	0.45
2	0.50
3	0.50

Raw Goat Milk.—Samples were collected from a local herd of Toggenburg goats. The copper content, as indicated in Table III, is much lower than that of cow's milk.

Processed Cow's Milk.—The copper content of milk that has been passed through a manufacturing process may vary between wide limits as shown in Table IV. The samples were analyzed

TABLE III.

Copper Content of Raw Whole Goat Milk.

Animal No.	Copper.
	mg. per l.
1	0.22
2	0.20
3	0.19
4	0.25
5	0.20
6	0.19

TABLE IV.

Copper Content of Processed Milk.

Product.	Factory No.	Sample No.	Copper.
			mg. per l.
Pasteurized whole milk.	I	1	0.60
		2	0.70
		3	0.63
	II	1	1.60
		2	1.50
		3	1.60
Buttermilk.	II	1	2.50
		2	2.45
		3	2.40
Concentrated milk.	III	1	2.32
	IV	1	1.80
	V	1	1.80
	VI	1	2.70

as described above. The concentrated milk samples were four common brands of condensed or evaporated milk. In all cases the copper was greater than could likely be accounted for in the

original raw product. The variation in copper content can be traced to the amount of copper surface exposed to the milk product.

BIBLIOGRAPHY.

1. Fluerent, E., and Levi, L., *Bull. Soc. chim.*, 1920, xxvii, 441.
2. Supplee, G. C., and Bellis, B., *J. Dairy Sc.*, 1922, v, 455.
3. Rice, F. E., and Miscall, J., *J. Dairy Sc.*, 1923, vi, 26.
4. Hess, A. F., Supplee, G. C., and Bellis, B., *J. Biol. Chem.*, 1923, lvii, 725.
5. Bertrand, G., *Bull. soc. hyg.-aliment.*, 1920, viii, 49.
6. Titze, C., and Wedemann, W., *Arb. k. Gsndhtsamte.*, 1911, xxxviii, 125.
7. McHargue, J. S., *Ind. and Eng. Chem.*, 1927, xix, 274.

ON THE QUESTION OF THE SPECIFICITY OF THE INTRACELLULAR DEHYDROGENASES.

I. THE DEHYDROGENASE OF CUNNER MUSCLE.

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(Received for publication, May 24, 1928.)

INTRODUCTION.

When food substances are burned in the tissues, the process may be one of direct addition of active oxygen to the food molecule, or of oxidation through the withdrawal of hydrogen from the food substance and its subsequent union with oxygen. The latter method was first discussed by Wieland (7) and later demonstrated by Thunberg and his school in a long series of papers which have been reviewed briefly by Hopkins (3) and in detail by Ahlgren (1). All tissues thus far tested contain a dehydrogenase (or dehydrogenases) capable of transferring hydrogen from certain donators to methylene blue, oxygen, or other receptors. The most complete observations have been made upon frog tissues, but considerable work has been done upon the tissues of the guinea pig and the muscle of man, rabbit, horse, dove, snake, goldfish, lobster, certain seed plants, and *Bacillus coli*.

The question at once arises as to whether all the donators are attacked by a single dehydrogenase, or whether each one has its own specific enzyme. Thunberg and his school have certain evidence pointing toward the latter possibility. Thus, the crystalline lens of the eye (guinea pig) attacks several donators but does not affect succinic acid, a donator readily attacked by other tissues (2). In the aging of sterile human muscle, power to attack lactic acid falls rapidly on the 1st day and then holds firm, power to attack citric acid is gradually diminished from the 1st day onward, while power to attack succinic acid is little affected in 5 days (5). After exposure to extremes of temperature ($-70°$ and $+60°$)

power to attack succinic acid is less affected than power to attack citric acid (6). Again grinding the tissue destroys its power to oxidize citric acid but does not greatly diminish its power to oxidize succinic acid (1). Extracts thus far made are rich in succinic dehydrogenase, but attack other donators less readily than the tissue itself. Perhaps the best evidence for Thunberg's theory of specificity is found in the fact that when frog muscle is added to a mixture containing optimal amounts of two donators (succinic and lactic, or succinic and glycerophosphoric acids), the reaction is more rapid than if only one of the donators is present, a result difficult to interpret unless there are at least three dehydrogenases (1).

The opposite point of view is taken by Wishart (8) who tested the decolorization time of a muscle extract upon optimal amounts of donators used singly and in pairs. Wishart observed no indication of an additive effect, but the value of his observations is lessened by the fact that he worked with a rather uncertain extract instead of with the muscle itself. Quastel and Wooldridge (4) in a long series of experiments upon *Bacillus coli* also conclude that, at least in that organism, there is only one dehydrogenase and that one not specific. It is of course possible that the dehydrogenase system is not identical in all organisms.

Evidently the solution of the problem demands further evidence. It is therefore proposed to test the question in various ways on the muscles of two organisms, fish and frog. In this paper experiments upon the muscle of the cunner (*Tautogolabrus adspersus*) are reported, in which optimal concentrations of various donators were used singly and in pairs, and the time measured in which a given quantity of methylene blue was completely decolorized *in vacuo*. If there is only one dehydrogenase, the decolorization time with a pair of donators will be no shorter than with the more active of them singly. If there are several specific dehydrogenases, each attacking a special donator, the decolorization time must be shorter when the donators are used in pairs, for the two reactions will proceed independently and the effect will therefore be additive.

Method.

In general the standard method used by Thunberg was followed (1,6). The fish were stunned by a blow, decapitated, and skinned.

The heavy dorsal trunk muscles were removed, cleared of the bones. The muscle mass, weighing some 10 gm., was finely divided by cutting for 15 minutes with a curved scissors. It was then shaken twice, for 15 minutes each time, with 20 times its weight of redistilled water in order to remove the donators and excess salts. After each washing it was filtered on cheese-cloth and perfused with 50 cc. of K_2HPO_4 0.075 M. The use of a buffer rinse is a departure from the usual method and is useful in maintaining the activity of the enzyme at a high level. The washed tissue was kept in a covered watch-glass in the refrigerator until just before use.

Stock solutions of the reagents (except methylene blue) were made daily with fresh, redistilled water. The content of each tube was as follows:

0.05 cc. methylene blue 1:5000.
0.15 " K_2HPO_4 0.5 M.
0.10 " donator 0.1 M (neutralized with KOH).
(0.10 " " 0.1 ") " " "
0.60 " H_2O.
1.0 " total fluid.
0.2 gm. washed muscle.

Each tube was evacuated as soon as the muscle had been added. Evacuation requires 30 to 60 seconds with a water pump if the pressure is above 50 pounds per square inch. It is important to continue the evacuation until all bubbles of gas are removed from the tissues. The tube is continually tapped on a cork block to keep the tissues from sticking to the walls and to prevent foaming. When warming the tube in one's hand no longer leads to the appearance of bubbles, evacuation is complete.

The time was noted when the tube was placed in the bath and again when decolorization was complete. The temperature was kept at 36°. The bath is essentially like the one described by Ahlgren (1), but differs from his in having the stirrer suspended from above the center of the bath and in being heated by blackened carbon bulbs. The arrangement was worked out by the apparatus department of the Marine Biological Laboratory at Woods Hole.

TABLE I.

Decolorization Time, in Minutes, of Donators Alone and in Pairs.

Acid.					
Aug., 1926.	17	29	30	19	15
Citric	45	23	43		
" and succinic	31	15	16		
Succinic	30	16	13		
" and glycerophosphoric	44	20	17		
Glycerophosphoric	80	40	34		
Citric and glycerophosphoric	67	27	32		
"	63	23	44	45	59
Lactic and citric	47			37	66
"	45			23	59
" and succinic	40			22	50
Succinic	40			22	39
Lactic and glycerophosphoric	56				60
Glycerophosphoric	56			28	60
Acetic	99			34	73
" and citric	73			55	70
Citric	60			42	70
Acetic and succinic				23	
Succinic	42			24	
Acetic and glycerophosphoric	78			40	
Glycerophosphoric	78	39	49	35	
" and *l*-malic		41	57		
l-Malic		38	58		
" and citric		31	48		
Citric		31	47		
l-Malic and succinic		18	15		
Succinic		19	13		

TABLE II.

Effect of Buffer Rinse upon Decolorization Time, in Minutes.

Acid.	6 hrs. after washing.	
	Rinsed.	Not rinsed.
Succinic	25	38
Glycerophosphoric	60	80
Citric	70	100

Results.

Table I shows typical results. It is evident at once that when two donators at optimal concentration are combined, the decolorization is not any quicker than when the more active of the two is used alone. There is consequently no evidence whatever of an additive effect. In the single experiment performed with acetic and butyric acids, the same result was observed. The effect of the buffer rinse in keeping the activity of the tissue at a high level is shown in Table II.

CONCLUSIONS.

In cunner muscle a single dehydrogenase is present, which is capable of oxidizing anaerobically several donators; *viz.*, succinic, citric, glycerophosphoric, lactic, *l*-malic, acetic, and butyric acids.

Rinsing the chopped muscle in the buffer after washing it in distilled water improves the activity of the enzyme.

BIBLIOGRAPHY.

1. Ahlgren, G., *Skand. Arch. Physiol.*, 1925, xlvii, suppl.
2. Ahlgren, G., *Skand. Arch. Physiol.*, 1923, xliv, 196.
3. Hopkins, F. G., *Skand. Arch. Physiol.*, 1926, xlix, 33.
4. Quastel, J. H., and Wooldridge, W. R., *Biochem. J.*, 1927, xxi, 148.
5. Rosling, E., *Skand. Arch. Physiol.*, 1924, xlv, 132.
6. Thunberg, T., *Skand. Arch. Physiol.*, 1920, xl, 1.
7. Wieland, H., *Ber. chem. Ges.*, 1912, xlv, 484, 2606.
8. Wishart, G. M., *Biochem. J.*, 1923, xvii, 103.

STUDIES IN THE METABOLISM OF THE BILE.

II. THE SEQUENCE OF CHANGES IN THE BLOOD AND BILE FOLLOWING THE INTRAVENOUS INJECTION OF BILE OR ITS CONSTITUENTS.*

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Knowledge of the physiologic mechanisms concerned in the formation and excretion of the bile is essential to the understanding of changes observed clinically in diseases of the liver or biliary tract. This is particularly true in the study of those conditions in which there is retention or accumulation of biliary constituents in the blood. In such conditions the institution of suitable treatment must be based on an understanding of the manner in which these materials are normally removed from the blood stream. We have sought, therefore, to provide an experimental basis for future clinical study by an investigation of the sequence of changes in the blood and bile following the intravenous injection, both of bile and of its two chief constituents, the bile acids and bilirubin.

The rapid disappearance of bile or bile salts from the blood stream after their intravenous injection was noted by Kühne (15) and Huppert (13). The methods at their disposal, however, did not permit detailed study of the process. The accurate study of changes in the bilirubin content of the blood has been made possible by the work of van den Bergh (2) in the development of the test that bears his name. The work of Aldrich (1) on a quantitative modification of the Pettenkofer reaction applicable to the analysis of small quantities of blood permits us to estimate the bile acids in a similar fashion.

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We have used these newer methods to study the elimination of bile or its constituents following their intravenous injection. Two series of experiments were carried out. The first was planned to compare the rate at which bile acids, bilirubin, and bile disappeared from the blood stream and to determine the effect of varying doses. The second was planned to compare the rate at which the bile acids and bilirubin were eliminated in the bile and the effect of each on the volume and composition of the latter.

Method.

The bile used for injection was obtained from hospital patients at operation. The absence of infection in such specimens was shown by the associated bacteriologic studies of Judd, Mentzer, and Parkhill (14). Purified samples of bilirubin were obtained from the Eastman Kodak Company. Commercial samples of bile salts, obtained from Armour and Company, which are mixtures of sodium glycocholate and taurocholate were used. These were analyzed as described by Aldrich and Bledsoe (1) and the dosage given was calculated on the basis of the content of bile acids.

Dogs were used in all experiments. In the study of the changes in the blood, the desired amount of bile, bilirubin, or bile salts, calculated in terms of mg. for each kilo of body weight, was dissolved in a physiologic solution of sodium chloride and injected intravenously at a uniform rate by means of a modified Woodyatt pump. A period of 1 hour was used for the injections which were made into the saphenous vein under local anesthesia. Specimens of blood for analysis were withdrawn from the jugular vein at the beginning of the experiment. Subsequent samples were taken during the period of injection and the course of recovery. The amounts given, while large in proportion to the usual daily output of a dog with a biliary fistula, were usually well tolerated. Some animals were restless and vomited during the injection, but recovery was complete by the following day. The largest doses, however, were toxic and caused death of the animal in from 12 to 48 hours.

The study of the composition of the bile was carried out on dogs under amytal narcosis. The gallbladder was removed and a cannula placed in the common bile duct. Samples of the hepatic

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TABLE I.

Elimination of Bile Acids from Blood Following Timed Intravenous Injection.

Experiment No.	Date.	Dose.	Normal before injection.	Injection period, min.						Elimination period, min.								
				10	20	30	40	50	60	10	20	40	60	80	100	120	150	
	1926	mg. per kg.	Glycocholic acid, mg. per 100 cc.															
1	Feb. 3	75	4.8	4.5	5.2	4.8	5.6	5.6	5.8	7.8	5.1	6.0	4.3		4.8	4.4	4.8	
2	" 10	150	4.3	4.6	9.2	10.5	10.6	11.8	12.1		5.4		5.6	5.4	4.4	4.8	4.3	
3	Mar. 25	250	7.6	17.6	23.0	23.0	28.7		43.5	19.1	10.0	10.2	8.7	8.2	8.8	7.8	8.9	
4	" 12	300	6.8	29.4	47.6	65.8	65.6	74.0	80.0	60.6	40.8	20.2	12.5	9.0	8.8	8.1	7.6	
5	Apr. 23	350	5.8	35.7	42.8	62.8	81.5	94.0	103.4	81.5	63.3	38.1	15.8	9.4	9.1	8.5	7.1	
6	Mar. 19	400	6.8	47.2	76.3		95.2	130.0	147.0	Died.								

bile so obtained were collected at half hour intervals. Usually two preliminary samples were collected to establish the normal rate of flow of the bile. Commercial preparations of bile salts were then injected intravenously in a dosage equivalent to from 100 to 125 mg. of glycocholic acid for each kilo of body weight and the collection of bile continued for a period of 6 hours. Samples of blood were taken from the jugular vein at appropriate intervals and the bile acids determined by the method of Aldrich (1). The concentration of bilirubin and bile acids in the specimens of bile was determined by the methods previously described (10).[1] Kühne (15), Huppert (13), Hoppe-Seyler (11), Brakefield and Schmidt (4), and others have reported the appearance of bile acids in the urine following their intravenous injection. To prevent such loss through the urine, both kidneys were removed at the beginning of the experiment. Control experiments were made on fasting animals and the effects of the injection of hemoglobin and bilirubin were studied for contrast with the action of the bile salts.

To control the effect of the anesthetic and of the operative procedures a second group of experiments was performed on a dog with a permanent biliary fistula. In the preparation of this fistula the gallbladder was removed and the common bile duct intubated according to the technique reported by Rous and McMaster (16). Samples were collected for analysis according to the same schedule as in the anesthetized dogs.

Rate of Elimination of Bile and Its Constituents from Blood.

Bile Acids.—When the sodium salts of the bile acids are injected intravenously under the conditions of these experiments there is rapid increase in the intensity of the Pettenkofer reaction given by the blood. Varying amounts of material equivalent to doses of from 75 to 400 mg. for each kilo were given. In all cases the dosage was considerably in excess of the usual production

[1] Glycocholic acid was used as a standard in the determination of the bile acids and the results are reported in terms of mg. of glycocholic acid for each 100 cc. of blood or in per cent of the bile. Dog bile normally contains only taurocholic acid. We did not attempt to differentiate between the two. The figures obtained should be increased by 10 per cent if a comparison on the basis of taurocholic acid is to be made.

of bile acids in an animal, as measured by means of a biliary fistula. The smallest dose, 75 mg. for each kilo, caused little if any change in the blood. With larger doses (Table I) the bile acid content of the blood was markedly increased. The height of this increase varied with the dose given. As soon as the injection was stopped the excess was removed from the blood with great rapidity. In Experiment 5 the level in the blood was increased to 103.4 mg. for each 100 cc. but this was reduced to 8.5 mg. within 2 hours. When smaller doses were given the content of the blood was not so greatly increased and the normal level was regained in a shorter time.

TABLE II.

Elimination of Bile Acids from Blood Following Rapid Intravenous Injection.

Experiment No.	Date.	Dose.	Normal before injection.	Time after injection, min.									
				5	10	15	30	45	60	90	120	150	180
			Glycocholic acid, mg. per 100 cc.										
	1926	*mg. per kg.*											
7	May 20	40	5.3	14.6	6.9	6.4	7.8	6.8	6.8	6.3	6.0	5.0	4.5
8	" 17	80	5.4	38.4	25.1	11.0	5.9	5.8	5.9	5.0	5.3	4.8	5.2
9	" 21	120	5.2	37.5	28.1	20.4	7.4	4.8	5.4		4.8	5.2	6.2
10	" 13	160	5.0	72.5	64.0	61.6	32.4	14.5	8.2	5.8	5.2	5.1	5.1
11	" 31	200	7.3	87.0	75.4	63.7	42.8	10.1	9.5	7.1	8.4	8.4	7.1
12	June 1	240	7.3	146.0	126.0	120.0	102.0	70.0	42.0	13.3	9.0	8.0	7.8

For purposes of comparison a similar group of experiments was made in which the bile acids were given at a more rapid rate. A 5 per cent solution was used and amounts of from 40 to 240 mg. for each kilo were injected within from 2 to 3 minutes. The changes in the bile acid content of the blood in these experiments are shown in Table II. As with the first group of experiments the increase of bile acids in the blood was dependent on the dose given. With equivalent doses, the shorter period of injection produced a much greater increase in the level in the blood. In Experiment 12 a level of 146 mg. for each 100 cc. of blood was reached after the injection of 240 mg. for each kilo of body weight. The disappearance of the excess of bile acids from the blood was

TABLE III.

Elimination of Bilirubin from Blood Following Timed Intravenous Injection.

Experiment No.	Date.	Dose.	Normal before injection.	Injection period, min.				Elimination period, min.									
				15	30	45	60	15	30	45	60	90	120	150	180	210	240
	1927	mg. per kg.	Bilirubin, mg. per 100 cc. serum.														
13	Jan. 17	10	0.0	0.6	1.2	1.7	2.4	1.2	0.6	0.3	0.0	0.0	0.0	0.0	0.0		
14	" 20	25	0.0	2.3	4.1	4.1	5.3	3.6	2.9	2.2	1.6		0.9	0.8	0.8	0.7	0.3
15	Mar. 9	35	0.0	6.1	8.8	11.2	17.4	17.0	16.0	15.5	14.2	12.8	12.7	11.9	10.1		
16	Feb. 14	50	0.0	10.4	15.3	20.0	23.1	22.1	18.5	17.2	16.5	11.7	9.7	7.4	5.7	4.8	4.3

quite as rapid as in the first experiments with slower injections and the concentration was reduced to 8 mg. 2½ hours later. The smaller doses produced less marked increases in the concentration and the excess was removed in a correspondingly shorter time.

In the experiments dealing with the excretion of bile acids in the bile, a standard dose of bile acids equivalent to 125 mg. of glycocholic acid for each kilo of body weight was rapidly injected. This produced an approximately tenfold increase in the blood over the normal Pettenkofer reading of from 5 to 6 mg. for each 100 cc. The disappearance of the excess was rapid, and, with the dosage used, the level in the blood decreased to one-half or one-third of the maximum in 30 minutes. The normal value was regained approximately by the end of the hour. In one experiment, samples of blood were taken from the portal vein. There was no significant difference between the amount of bile acids in this blood and in samples from the systemic circulation. We were unable to demonstrate bilirubinemia by means of the van den Bergh reaction in any of these experiments.

Bilirubin.—Bilirubin accumulated in the blood during its intravenous injection in much the same manner as the bile acids. Amounts varying from 10 to 50 mg. for each kilo of body weight were given. The effect varied with the dose. As shown in Table III, the injection of 10 mg. for each kilo of body weight resulted in a concentration of 2.4 mg. for each 100 cc. of serum, while a level of 23.1 mg. was attained after the administration of 50 mg. for each kilo of body weight. The excess of bile pigment readily left the blood stream although at a much slower rate than the bile acids. Considerable amounts of bilirubin were present in the serum 4 hours after the injection in all the experiments, except the one in which the smallest dose, 10 mg., was used. The curves of bilirubin elimination showed more variation than was found in the experiments with the bile acids. These variations were seemingly related to the general condition of the animal.

The injection of bilirubin alone was without effect on the intensity of the Pettenkofer reaction of the blood. The readings for the bile acids were unchanged during these experiments.

Mixtures of Bile Acid and Bilirubin.—One experiment (Table IV, Experiment 17) was made in which a mixture of bilirubin and bile salts was injected. In this experiment the increase in the

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TABLE IV.

Elimination of Bile Acids and Bilirubin from Blood Following Timed Intravenous Injection of a Mixture or of Whole Bile.

Experiment No.	Date.	Material injected.	Content of material injected.	Dose.	Normal before injection.	Injection period, min.				Elimination period, min.									
						15	30	45	60	15	30	45	60	90	120	150	180	210	240
					Mg. per 100 cc. of blood or serum.														
	1927			*mg. per kg.*															
17	Apr. 16	Mixture of pure material.	Bile acids.	200	4.8	7.1	9.1	10.2	15.4	7.0	5.8	5.3	5.2	5.3	5.1	4.9		5.0	4.9
			Bilirubin.	25	0.0	3.0	3.6	4.7	5.9	4.0	3.9	3.2	2.3	2.1	1.6	1.3	0.5	0.7	0.3
18	Jan. 12	Whole bile.	Bile acids.	110	5.3	10.1	10.5	11.0	11.5	7.0	6.9	6.1		6.0	5.2	5.1	5.1		4.7
			Bilirubin.	5	0.0	0.8	1.3	1.5	1.9	1.0	0.8	0.7	0.7	0.3	0.0	0.0	0.0	0.0	0.0
19	Feb. 1	" "	Bile acids.	155	6.5	22.0	35.3	41.1	47.1	36.6	34.3	29.6	25.8	18.2	12.8	8.2	7.7	7.4	5.9
			Bilirubin.	15	0.0	2.2	3.8	5.1	5.8	5.1	4.9	4.8	4.2	3.8	3.0	2.7	1.7	0.6	0.0
20	Jan. 15	" "	Bile acids.	175	7.5	26.3	38.7	49.4	57.5	48.0	46.5	40.0	40.8	32.1	20.0	19.2	18.0	16.9	16.7
			Bilirubin.	15	0.0	3.9	5.7	7.9	9.1	7.5	7.3	7.0	6.9	6.7	5.4	5.4	5.3	4.9	4.8
21	" 18	" "	Bile acids.	200	7.0	36.0	63.0	63.0	70.0		62.4	55.0	54.6	53.2	50.0	48.0	45.6	45.3	42.5
			Bilirubin.	10	0.0	2.3	3.3	4.3	5.2	4.2	4.1	4.1	3.6	4.2	4.1	3.9		3.3	
22	Feb. 18	" "	Bile acids.	200	9.0	64.5	92.8	102.0	103.0	98.0	85.1	78.1	71.5	54.0	39.0	29.2	20.0	18.7	
			Bilirubin.	25	0.0	6.5	11.1	12.0	13.2	12.5	11.4	10.2	9.3	8.8	8.6	7.8	6.5	5.3	

concentration in the blood and the curve of excretion of each constituent of the mixture was essentially the same as when each was given alone.

Whole Bile.—Whole bile was diluted with a physiologic solution of sodium chloride and injected in the same manner as the solutions of bile salts or bilirubin. The changes observed, as recorded in Table IV, differ considerably from those that followed the injection of purified materials.

Bile containing an amount of bile acids equivalent to 200 mg. for each kilo of body weight increased the amount in 100 cc. of blood to 103 mg. in one instance (Experiment 22) and to 70 mg. in another (Experiment 21). Furthermore elimination was greatly delayed and the levels were 18.7 and 45.3 mg., respectively, at the end of 3½ hours. The same dose of bile salts in pure solution raised the reading in the blood to but 26.1 mg. and normal levels were regained in less than 2 hours. Similar results, although less striking, were obtained by the injection of bile containing smaller amounts of bile acids.

Analogous changes were observed in the behavior of the bile pigment. In Experiment 22, bile containing 25 mg. of bilirubin for each kilo of body weight was injected. This raised the level in the blood to 13.2 mg., and 5.3 mg. were still present at the end of 4 hours. The same dose of bilirubin when given alone (Table III, Experiment 14) provided a maximal concentration in the serum of 5.3 mg., and only 0.3 mg. was present at the end of the experiment. Similar results were obtained in the other experiments in which bile was injected.

Composition of Bile after Intravenous Injection of Certain of Its Constituents.

Bile salts given intravenously have a marked cholagogue effect. The increased rate of bile flow could be noted within 15 minutes although the maximal effect was present during the second collecting period (Tables V and VI). This maximal increase varied from 4 to 10 times the normal, the most marked change being observed in Experiment 23 (Table V) in which 15 cc. (1.1 cc. of bile for each kilo of body weight) were obtained during one 30 minute collection period. The period of maximal choleresis was of short duration and did not last more than 2 hours, although the bile flow

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was usually somewhat augmented over the normal during the entire course of the experiment. On the other hand, in control experiments on fasting animals in which salt solution only was injected (Table VII), the bile flow decreased slightly during the

TABLE V.

Changes in Blood and Bile Following Intravenous Injection of Bile Acids.

Experiment 23. Weight of dog 13.3 kilos.

Time of collection.		Blood.		Bile.					Remarks.
Before injection.	After injection.	Bile acids.		Volume.	Bilirubin.		Bile acids.		
		Jugular.	Portal.			Total.		Total.	
hr.	*min.*	*mg. per cent*	*mg. per cent*	*cc.*	*mg. per cent*	*mg.*	*per cent*	*mg.*	
1		6.9		1.1	80	0.88	4.20	47	Control period.
½		6.1	5.7	1.9	72	1.37	3.05	58	
	5	64.0							52 cc. bile salt solution containing 1540 mg. bile acids injected intravenously. Total bile acids recovered 1885 mg., total bilirubin 28.59 mg.
	10	50.8	52.0						
	15	38.2							
	30	21.4	24.8	11.0	16	1.76	4.33	480	
	45	14.2							
	hrs.								
	1	8.9	7.5	15.0	13	1.95	4.84	730	
	1½	7.7		8.5	43	3.65	2.62	222	
	2	6.7	7.7	4.4	88	3.87	1.90	84	
	2½			3.5	96	3.36	1.90	67	
	3	5.5		3.4	100	3.40	1.90	65	
	3½			2.5	108	2.70	2.25	56	
	4	5.6	7.6	2.2	110	2.42	2.40	53	
	4½			2.0	111	2.22	2.97	59	
	5	6.2		1.3	144	1.82	2.97	39	
	5½			0.5	144	0.72	2.97	15	
	6	4.8	5.7	0.5	144	0.72	2.97	15	

first two periods but was relatively constant thereafter. This decrease, first observed by Schiff (17), is associated with the external drainage of the bile and consequent interruption of the normal enterohepatic circulation of the bile acids.

The concentration of bile acids in the bile increased but to a much less degree than the increase in volume of the bile secreted. The concentration in the normal hepatic bile obtained during the preliminary periods varied from 1.80 to 6.04 per cent. After the

TABLE VI.

Changes in Blood and Bile Following Intravenous Injection of Bile Acids.

Experiment 24. Weight of dog 12.2 kilos.

Time of collection.		Blood.	Bile.					Remarks.
Before injection.	After injection.	Bile acids.	Volume.	Bilirubin.		Bile acids.		
					Total.		Total.	
hr.	min.	mg. per cent	cc.	mg. per cent	mg.	per cent	mg.	
1			1.0	117	1.17	4.9	49	Control period.
½		6.3	0.6	117	0.70			
	5	56.1						46 cc. bile salt solution containing 1400 mg. bile acids injected intravenously. Total bile acids recovered 1925 mg., total bilirubin 39.79 mg.
	10	50.6						
	15	38.9						
	30	22.4	9.3	23	2.14	5.95	554	
	45	11.5						
	hrs.							
	1	10.9	12.6	16	2.02	4.65	586	
	1½	6.8	5.4	46	2.48	4.10	221	
	2	6.1	4.4	77	3.38	3.53	155	
	2½		2.6	113	2.94	3.54	92	
	3	5.8	2.3	137	3.15	2.02	46	
	3½		2.1	147	3.09	1.57	33	
	4	5.7	4.3	134	5.75	1.18	51	
	4½		4.6	132	6.06	1.11	51	
	5	5.5	4.3	68	2.92	1.02	44	
	5½		4.6	62	2.85	1.00	46	
	6	5.4	4.6	64	2.94	1.00	46	

injection of bile salts the maximal values varied from 4.33 to 6.00 per cent. This maximal value was attained during the 1st hour; later the concentration of bile acids diminished and minimal values of 1.00 to 2.94 per cent were reached during the latter

portion of the experiment. Similar changes in concentration were observed during the control experiments.

The effect of the intravenous injection of the bile acids on their excretion in the bile is best shown when the total quantity rather than the concentration is studied. During the preliminary

TABLE VII.

Changes in Blood and Bile in Control Experiment.

Experiment 25. Weight of dog 11.1 kilos.

Time of collection.		Blood.	Bile.					Remarks.
Before injection.	After injection.	Bile acids.	Volume.	Bilirubin.		Bile acids.		
					Total.		Total.	
hr.	*min.*	*mg. per cent*	*cc.*	*mg. per cent*	*mg.*	*per cent*	*mg.*	
1		1.0	0.7	173	1.21	4.45	31	Control period.
½		5.0	0.3	183	0.55	4.50	13	
	5	4.4						35 cc. physiologic solution of sodium chloride injected intravenously. Total bile acids 209 mg., total bilirubin 15.80 mg.
	10	4.5						
	15	4.2						
	30	5.0	0.2	311	0.61	5.00	10	
	45	5.0						
	hrs.							
	1	5.2	0.4	315	1.26	6.40	25	
	1½	5.3	0.4	294	1.18	5.08	20	
	2	4.3	0.4	375	1.50	4.10	16	
	2½		0.4	379	1.50	4.80	19	
	3	4.5	0.3	400	1.20	5.40	16	
	3½		0.4	365	1.45	5.10	20	
	4	4.4	0.5	433	2.16	5.17	26	
	4½		0.4	390	1.56	4.45	18	
	5	4.2	0.5	320	1.60	2.78	14	
	5½		0.5	276	1.38	2.82	14	
	6		0.4	98	0.40	2.82	11	

periods of the various experiments, the amount varied from 13 to 60 mg. (1.2 to 4.4 mg. for each kilo of body weight) for each 30 minute period depending on the condition of the animal. In the control experiments the output of bile acids was greatest during the first two periods (Table VII, Experiment 25). There-

TABLE VIII.

Changes in Blood and Bile of Animal with Permanent Biliary Fistula.

Time of collection.		Blood.	Bile.					Remarks.
Before injection.	After injection.	Bile acids.	Volume.	Bilirubin.		Bile acids.		
					Total.		Total.	
Experiment 28. Weight of dog 14.6 kilos. Changes following the intravenous injection of bile acids.								
hr.	*min.*	*mg. per cent*	*cc.*	*mg. per cent*	*mg.*	*per cent*	*mg.*	
1		6.1	7.4	16	1.17	2.34	173	Control period.
½		6.0	6.5	21	1.42	1.28	83	
	5	37.2						46 cc. bile salt solution containing 1460 mg. bile acids injected intravenously. Total bile acids recovered 1304 mg.
	10	32.0						
	15	21.3						
	30	10.1	15.1	14	2.08	3.60	540	
	45	7.3						
	hrs.							
	1	6.6	8.5	20	1.66	2.42	206	
	1½	6.5	7.3	38	2.75	1.55	113	
	2	6.5	6.3	48	3.00	1.02	64	
	2½		6.2	71	4.43	0.93	58	
	3	6.1	6.1	54	3.30	0.88	54	
	3½		4.8	60	2.90	0.87	42	
	4	6.3	5.1	62	3.20	0.87	44	
	4½		5.3	56	3.00	0.91	48	
	5	5.9	5.0	61	3.05	0.92	46	
	5½		5.0	61	3.00	0.92	46	
	6	5.7	4.6	56	2.60	0.94	43	
Experiment 29. Weight of dog 14.6 kilos. Control experiment.								
1			13.0	40	5.20	1.63	181	Control period.
½		6.7	8.0	44	3.50	1.39	111	
	½		8.2	67	5.55	1.00	82	29 cc. salt solution injected intravenously. Total bile acids 627 mg.
	1	6.5	7.8	91	7.10	0.76	59	
	1½		7.6	68	5.15	0.59	45	
	2	6.7	7.5	63	4.70	0.57	43	
	2½		8.0	58	4.60	0.56	45	
	3	6.5	8.3	61	5.05	0.59	49	
	3½		8.9	56	4.95	0.56	50	
	4	6.5	7.9	57	4.50	0.55	44	
	4½		7.3	60	4.40	0.72	53	
	5	6.8	7.1	64	4.60	0.78	55	
	5½		7.0	57	3.95	0.76	53	
	6	6.3	7.3	65	4.75	0.72	53	

after it was constant or decreased gradually during the observation period, the final readings being one-half to one-third of the initial ones. Following the injection of bile salts, on the other hand, there was a marked increase in the total quantity excreted in the bile. Approximately 0.75 gm. was obtained in the second half hour specimen in Experiment 23 (Table V). This increase was for the most part confined to the first four collection periods after the injection. In the more satisfactory experiments sufficient bile acids to account for the whole of the amount injected were excreted during this 2 hour interval.

Similar results were obtained in the experiments on a dog with a permanent biliary fistula (Table VIII). Such animals consistently excrete a larger volume of bile than those operated on under anesthesia, but the concentration of bile acids is less. Apart from this difference, the physiologic response to the injection of bile salts was the same in both types of experiments. The recovery of the injected bile acids was slightly less complete in the animal with a permanent biliary fistula. However, qualitative tests showed the presence of bile acids in the urine and this renal loss will serve to explain the discrepancy.

A striking phenomenon in these experiments was the decrease in the intensity of the color of the bile during the periods of marked choleresis. The concentration of bilirubin in the bile during the preliminary periods varied from 54 to 186 mg. per cent. This concentration was promptly reduced to between 9 and 17 mg. following the injections, but the concentration gradually increased to a later maximum of from 56 to 147 mg. A consideration of the total quantity excreted shows that the normal rate of excretion was maintained during the 1st hour of the experiment, but thereafter, there was a gradual increase to 2 or 3 times that value between the 2nd and 5th hours. The excretion of bilirubin in the normal control was maintained at a relatively uniform rate throughout the experiment. The concentration in the bile increased slightly but the total quantity was constant apart from the fluctuations inherent in the collection and measurement of such small samples of bile.

Frerichs (7) observed the appearance of bilirubin in the urine after the intravenous injection of solutions of bile salts. He ascribed this to the transformation of bile salts into pigment. It

remained for Kühne (15) to point out that this extra pigment had its origin in hemoglobin set free in consequence of the hemolytic action of the bile acids. An additional control experiment (Table IX), was therefore performed in which the animal received 1 cc. of laked blood for each kilo of body weight. This in-

TABLE IX.

Changes in Blood and Bile Following Intravenous Injection of Laked Blood.

Experiment 26. Weight of dog 12.0 kilos.

Time of collection.		Blood.	Bile.					Remarks.
Before injection.	After injection.	Bile acids.	Volume.	Bilirubin.		Bile acids.		
					Total.		Total.	
hr.	*min.*	*mg. per cent*	*cc.*	*mg. per cent*	*mg.*	*per cent*	*mg.*	
1			0.70	117	0.82	6.04	42	Control period.
½		5.1	0.60	186	1.11	7.70	46	
	5	4.2						12 cc. laked blood injected intravenously. Total bile acids 211 mg., total bilirubin 42.88 mg.
	10	5.1						
	15	4.4						
	30	5.0	0.50	264	1.32	6.91	35	
	45	5.1						
	hrs.							
	1	5.0	0.75	275	2.06	4.04	30	
	1½	5.1	0.45	670	3.01	4.22	19	
	2	5.7	0.45	625	2.82	4.00	18	
	2½		0.30	876	2.62	3.64	11	
	3		0.25	1000	2.50		10	
	3½		0.55	910	5.50	3.85	21	
	4	5.7	0.45	880	3.96	2.50	11	
	4½		0.65	880	5.70	2.86	19	
	5	5.1	0.50	710	3.55	2.50	13	
	5½		0.70	710	4.95	2.00	14	
	6	4.8	0.40	690	2.76	2.50	10	

jection of hemoglobin produced a threefold to fourfold rise in the excretion of bilirubin between the 2nd and the 6th hours. The maximal increase occurred about the 4th hour. There was no increase in the flow of bile in this experiment; the volume output was small, and the specimens were extremely concentrated.

Bilirubin alone produced a somewhat similar result. In Experiment 5 bilirubin was injected intravenously in a dosage equivalent to 2 mg. for each kilo of body weight (Table X). The pigment left the blood rapidly although at a slower rate than the bile salts. The serum bilirubin was increased to 2.5 mg. 5

TABLE X.

Changes in Blood and Bile Following Intravenous Injection of Bilirubin.

Experiment 27. Weight of dog 17.5 kilos.

Time of collection.		Blood.	Bile.					Remarks.
Before injection.	After injection.	Bilirubin.	Volume.	Bilirubin.		Bile acids.		
					Total.		Total.	
hr.	*min.*	*mg. per cent*	*cc.*	*mg. per cent*	*mg.*	*per cent*	*mg.*	
1		0.0	0.70	35	0.25	3.35	23	Control period.
½		0.0	0.45	45	0.20	3.20	14	
	5	2.5						35 cc. solution containing 35 mg. bilirubin injected intravenously. Total bilirubin recovered 59.47 mg., total bile acids 615 mg.
	10	2.3						
	15	1.7						
	30	1.1	0.25	36	0.09	3.60	9	
	45	0.7						
	hrs.							
	1	0.6	0.60	120	0.72	6.55	40	
	1½	0.5	0.75	320	2.40	6.60	49	
	2	0.3	1.60	820	13.10	7.90	126	
	2½	0.2	1.80	1050	18.90	8.35	150	
	3	0.1	0.75	950	7.10	8.30	62	
	3½	0.0	0.45	840	3.80	8.40	38	
	4	0.0	0.45	700	3.15	7.40	33	
	4½	0.0	0.65	700	4.55	7.65	50	
	5	0.0	0.30	640	1.92	7.25	22	
	5½	0.0	0.30	645	1.94	6.10	18	
	6	0.0	0.30	600	1.80	6.10	18	

minutes after the injection. 2 hours later it had fallen to 0.3 mg. This curve of elimination from the blood is similar to curves reported in the first section of this paper. The maximal excretion in the bile came in the fourth, fifth, and sixth half hour periods after the injection. The bilirubin was injected in 0.1 per cent

solutions, but during these periods the bile contained approximately 1.0 per cent of bilirubin, a concentration 20 times that in the initial control specimens and 10 times that of the bile originally obtained from the gallbladder of the same dog. In this experiment, there was an increase in both the concentration and volume of the bile, hence there was a very great increase in the total excretion of pigment. The total quantity of bilirubin injected was recovered in the bile during the experimental period.

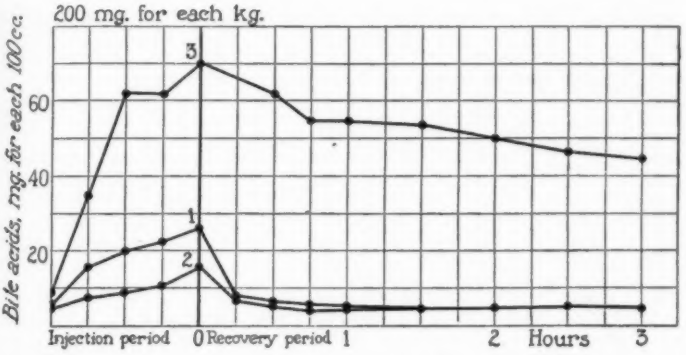

FIG. 1. Comparison of the changes in the bile acid content of the blood following the timed intravenous injection of (1) bile acids alone, (2) a mixture of bile acids and bilirubin, and (3) whole bile. The same dose of bile acids, 200 mg. for each kilo of body weight, was given in each experiment.

DISCUSSION.

The results obtained emphasize the independence in the physiologic behavior of bile acids and bilirubin. The increase in their concentration in the blood after the intravenous administration of bile or its constituents was determined by the dosage of each and the speed of injection. The bile acids are eliminated rapidly. Even the maximal dosage compatible with recovery of the animal, increases the concentration in the blood for only an hour or two. Injected bilirubin, on the other hand, leaves the blood at a fairly uniform rate over a period of several hours.

The experiments suggest that whole bile is more toxic than

either of its major constituents, a conclusion at variance with that of Horrall (12). Unfortunately there is no mention of dosage in his report so that it is difficult to compare the two series of experiments. The differences in the experimental technique may also serve to explain this discrepancy. We found that the injection of whole bile produced greater increases in the amount of bile acids in the blood, and the excess is more slowly removed from the blood stream, than when equivalent amounts are injected in pure solution (Fig. 1). Similarly, the injection of bilirubin solutions causes a lesser increase in the serum content and the excess is

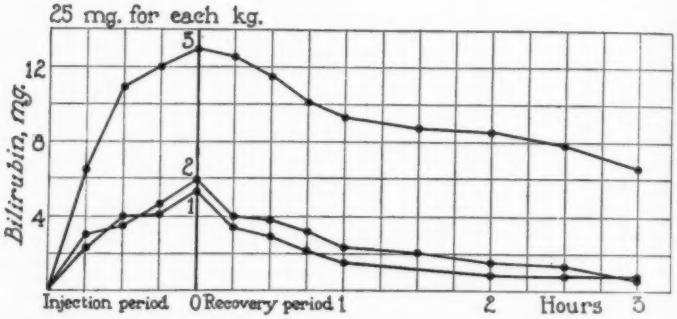

FIG. 2. Comparison of the changes in the serum bilirubin following the timed intravenous injection of (1) bilirubin alone, (2) a mixture of bilirubin and bile salts, and (3) whole bile. The same dose of bilirubin, 25 mg. for each kilo of body weight, was given in each experiment.

removed from the blood stream more rapidly than when bile containing an equivalent amount of pigment is given (Fig. 2). The elimination of these substances also may be greatly prolonged by various conditions which presumably affect the liver. This emphasizes the possibility of using injections of bile salts or bilirubin as tests for hepatic function. The clinical use of bilirubin has been reported by von Bergmann (3) and Eilbott (5), while studies on the elimination of the bile acids in experimental obstructive jaundice have been reported by Snell, Greene, and Rowntree (18). The curves showing the elimination of intravenously injected bile acids emphasize the rapidity of this process.

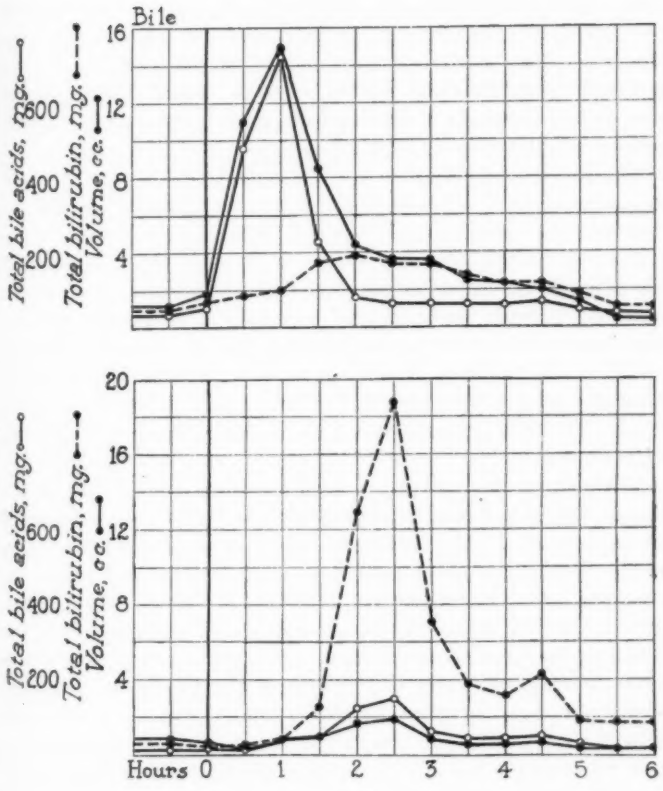

FIG. 3. Comparison of the changes in the volume and composition of the bile following the intravenous injection of bile acids and bilirubin. The upper chart shows the changes following the injection of bile acids in Experiment 23. The lower chart shows the changes following the injection of bilirubin in Experiment 27. The volume of the bile and the excretion of bile acids and bilirubin are shown. Two half hour collections of bile were taken in all experiments preceding the injection.

Their appearance in the bile follows the disappearance from the blood, with only a slight lag dependent on the rate of excretion by the liver. This depends in part on the condition of the animal; but if operative shock is avoided, or if an animal with a permanent biliary fistula is used, all of the injected bile acids may be recovered in the bile within 2 hours.

The cholagogue action of the bile acids is well shown in these experiments (Fig. 3). They are excreted primarily by an increase in the volume of the bile and the greatest change in the latter corresponded with the excretion of the greatest amount of the bile acids. The concentration of the bile acids was likewise maximal at such times, though the degree of change in concentration was much less marked than that in volume. The increase in the volume of bile, however, persisted for a period after the total output of the bile acids returned to the normal level. The bile in consequence was more dilute in the late after period.

The intravenous injection of solutions of bile salts results in the destruction of a certain number of erythrocytes. This is signified by an increase in the excretion of bilirubin during the later stages of the experiment. Similar changes in the output of pigment are produced by the intravenous injection of hemoglobin. When pure bilirubin is injected intravenously it also disappears from the blood to be excreted in the bile. This process, however, takes place more slowly than in the case of the bile acids, although seemingly it is equally complete. The excretion of injected bilirubin takes place primarily by an increase in the concentration of the bile rather than by a change in the volume output (Fig. 3). In one experiment there was an increase in the amount of bile during the periods of maximal excretion of bilirubin, but the cholagogue effect was slight, especially when contrasted with the marked effect of the bile acids. When the excretion of bilirubin is less pronounced, as after the injection of hemoglobin, the volume of the bile is not increased.

These experiments serve to emphasize the independence of the excretion of the bile acids and of bilirubin in the bile. The maximal concentration of bile acids observed was about 6 per cent. Foster, Hooper, and Whipple (6) reported concentrations of from 7 to 9 per cent in some of their experiments on animals

with fistulas. They used the gallbladder in the construction of the biliary fistula and a further concentration by this viscus cannot be excluded in their experiments. These high concentrations, however, were obtained in experiments in which the output of bile was reduced after the ingestion of sugar. We observed a concentration of 8 per cent of bile acids following the intravenous injection of bilirubin (Table X). In this experiment a relatively small amount of concentrated bile was obtained. The usual output was more dilute. The bile obtained in experiments on dogs with permanent biliary fistulas was in general more dilute than that secured by the direct intubation of the common duct under anesthesia. Injury to the liver by obstruction to the fistula or by infection further reduces the attainable concentration of bile acids in the bile. Apparently the usual range of variation in the concentration of bile acids in the bile as secreted by the liver is relatively slight and an increase in the output of bile acids is primarily attained through the medium of an increase in the volume of the bile. In this respect the condition is analogous to the condition in chronic nephritis in which there is a loss of the concentrating activity of the kidney, and the excretion of urea can be augmented only by an increase in the volume of the urine.

The liver is normally able to secrete bile of a widely varying bilirubin concentration. These experiments suggest that concentrations of from 20 to 1000 mg. for each 100 cc. are still within the limits of the normal. An increased excretion of bilirubin in the bile is brought about primarily by an increase in the concentration of the bile pigment, although when excessive amounts are present there may be an additional increase in the volume output. The data do not furnish very good evidence that bilirubin is washed out by an increased output of bile. On the other hand, the experiments were performed on normal animals in which bilirubin is excreted as rapidly as it is formed.

Clinical observations suggest that in some cases of disease of the liver, the bile contains only small amounts of pigment and "white bile" of hepatic origin may be present. Furthermore, although increased concentration of pigment in the bile is a recognized feature of hemolytic icterus this of itself is not sufficient to prevent the retention of bilirubin and the development of

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jaundice. The importance of further study of the physiologic factors determining the excretion of bile acids and bilirubin under such pathologic conditions is obvious.

SUMMARY.

Whole bile, bilirubin, and the bile acids quickly disappear from the blood stream following their intravenous injection in dogs. The bile acids leave the blood rapidly. Even with maximal doses the excess is removed within 2 hours. Bilirubin, on the other hand, is eliminated at a somewhat slower rate. When whole bile, obtained at operation, is injected, there is a greater increase in the level of each constituent in the blood, and the excess leaves the blood less rapidly than if given in pure solution.

This study also presents the curves of excretion by the liver of intravenously injected bile acids and of bilirubin, and compares the simultaneous changes in the concentration in the bile with those in the blood.

Bile acids have a pronounced cholagogue action; it was greatest during the first 2 hours of these experiments but the increased flow of bile continued for a longer period. The intravenously injected bile acids were excreted primarily by an increase in the volume of the bile rather than by changes in its concentration. The total quantity of bile acids administered could be recovered in the bile within 2 hours.

Injections of bilirubin or hemoglobin increase the output of pigment in the bile. This augmented rate of excretion is brought about primarily by an increase in the concentration rather than by changes in the volume of the bile.

Excretion of injected bilirubin takes place more slowly than the excretion of the injected bile acids.

BIBLIOGRAPHY.

1. Aldrich, M., and Bledsoe, M. S., Studies in the metabolism of the bile. I. A quantitative Pettenkofer test applicable to the determination of bile acids in the blood, *J. Biol. Chem.*, 1928, lxxvii, 519.
2. van den Bergh, A. A. H., Der Gallenfarbstoff im Blute, Leiden, 1918, viii.
3. von Bergmann, G., Zur Funktionellen Pathologie der Leber insbesondere der Alkohol-ätiologie der Cirrhose, *Klin. Woch.*, 1927, vi, 776.

4. Brakefield, J. L., and Schmidt, C. L. A., Studies on the synthesis and elimination of certain bile components in obstructive jaundice, *J. Biol. Chem.*, 1926, lxvii, 523.
5. Eilbott, W., Funktionsprüfung der Leber mittels Bilirubinbelastung, *Z. klin. Med.*, 1927, cvi, 529.
6. Foster, M. G., Hooper, C. W., and Whipple, G. H., The metabolism of bile acids. III. Administration by stomach of bile, bile acids, taurine, and cholic acid to show the influence upon bile acid elimination, *J. Biol. Chem.*, 1919, xxxviii, 379.
7. Frerichs, F. T., A clinical treatise on diseases of the liver, London, 1860–61, i.
8. Greene, C. H., and Aldrich, M., Studies in the metabolism of the bile acids, *Am. J. Physiol.*, 1927, lxxxi, 480.
9. Greene, C. H., Aldrich, M., and Rowntree, L. G., Studies in the metabolism of the bile, *J. Physiol.*, 1927, lxiv, 7.
10. Greene, C. H., Snell, A. M., and Walters, W., Diseases of the liver. I. A survey of tests for hepatic function, *Arch. Int. Med.*, 1925, xxxvi, 248.
11. Hoppe-Seyler, F., Ueber die Anwesenheit von Gallensäuren im icterischen Harne und die Bildung des Gallenfarbstoffes, *Virchows Arch. path. Anat.*, 1862, xxiv, 1.
12. Horrall, O. H., The toxic substances in bile, *Proc. Inst. Med. Chicago*, 1927, vi, 254.
13. Huppert, H., Ueber das Schicksal des Gallensäuren im Icterus, *Arch. Heilk.*, 1864, v, 236.
14. Judd, E. S., Mentzer, S. H, and Parkhill, E., A bacteriological study of gallbladders removed at operation, *Am. J. Med. Sc.*, 1927, clxxiii, 16.
15. Kühne, W., Beiträge zur Lehre vom Icterus, *Virchows Arch. path. Anat.*, 1858, xiv, 310.
16. Rous, P., and McMaster, P. D., A method for the permanent sterile drainage of intraabdominal ducts, as applied to the common duct, *J. Exp. Med.*, 1923, xxxvii, 11.
17. Schiff, M., Gallenbildung abhängig von der Aufsaugung der Gallenstoffe, *Arch. ges. Physiol.*, 1870, iii, 598.
18. Snell, A. M., Greene, C. H., and Rowntree, L. G., Diseases of the liver. VII. Further studies in experimental obstructive jaundice, *Arch. Int. Med.*, 1927, xl, 471.

XUM

NOTE ON THE FRACTIONATION OF SERUM PROTEINS BY MEANS OF AMMONIUM SULFATE.

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We have adopted the method proposed by Van Slyke and Cullen (1) and modified by Petschacher, Berger, and Schretter (2), who removed the free ammonia of the albumin solution with a current of air instead of steam. We have further modified the method by aerating at 60–70°, and by the use of superoxol. Total nitrogen can be determined with sufficient accuracy in dilutions corresponding to 0.1 cc. of serum. Albumin plus non-protein nitrogen can be determined in dilutions corresponding to $\frac{1}{6}$ cc. of serum.

Total nitrogen is estimated in serum diluted tenfold with 0.9 per cent sodium chloride solution. 1 cc. of the dilution is digested with 2 cc. of concentrated sulfuric acid and 2.5 cc. of 2 per cent copper sulfate solution. (Practically the same figures for nitrogen are found if the oxidation is finished by addition of 0.2 cc. of superoxol, Merck.)

The globulin is precipitated by half saturation with ammonium sulfate in serum diluted threefold with distilled water. The ammonium sulfate is added under constant shaking and after short standing the mixture is centrifuged for about 30 minutes at a speed of 2500 R.P.M. The supernatant fluid is carefully pipetted and used for determination of albumin plus non-protein nitrogen. The ammonium sulfate is decomposed by magnesium oxide in 50 per cent alcohol, as suggested by Van Slyke. The liberated ammonia is carried off by a rather vigorous air current, washed free of ammonia, care being taken to push the aeration tube down as far as possible. The flasks are kept in a water bath of 60–70°. After 2 to 3 hours the ammonia is completely driven off. It is advisable to have the temperature of the water bath not higher than 70° to prevent the contents of the flasks

XUM

from drying out, for otherwise it becomes difficult to determine whether the ammonia has been removed completely. To the residue in the flasks a few cc. of distilled water, 2.5 cc. of 20 per cent copper sulfate, and 2 cc. of concentrated sulfuric acid are added, and the flasks are heated over a low flame until the contents appear to be in fine suspension; then the water is boiled off and as soon as white fumes begin to appear, 0.2 cc. of superoxol (Merck) are added; the mixture clears up after a few minutes.

TABLE I.

Solution used.	Calculated concentration, N per 100 cc.	No. of analyses.	Average concentration, N per 100 cc.	Deviation from average.	Deviations from calculated concentration.
	mg.		*mg.*	*per cent*	*mg. per 100 cc.*
Ammonium sulfate solution 2 cc. (1.508 mg. N).	75.42	3	75.17	+0.5, −0.1, −0.4	−0.25 (−0.3 per cent).
Urea solution 2 cc. (1.507 mg. N).	75.33	7	73.48	+0.7, +0.8, −0.8, −0.5, +0.4, +0.6, −0.4	−1.85 (−2.5 per cent).
Urea solution 25 cc. (18.83 mg. N).	75.33	4	74.12	+1, −1, +0.3, −0.3	−1.21 (−1.6 per cent).

The ammonium sulfate used was Merck's reagent quality, dried to constant weight at 105°. The urea used was Eimer and Amend's urea, pure, dried to constant weight over concentrated sulfuric acid. The estimations of urea were made on different days in duplicate or triplicate. Macro-Kjeldahl determinations were employed in the last four analyses.

(Without addition of superoxol the oxidation takes a few hours and needs much supervision.)

For determination of non-protein nitrogen the usual precipitation with tungstic acid is used.

We have used for distillation the apparatus suggested by Fuchs (3), with the modification of using a Hopkins trap and substituting a block tin coil and Pyrex tubing for the Jena glass tubing used by Fuchs.

To obviate repeated testing for the end-point of the distillation

it seems advisable to distil half the volume in the distilling flask over, starting with a total volume of about 40 cc. The distillation should be slow in the beginning and the tin coil should

TABLE II.

Protein Fractionation of Serum.

Sample No.	Total protein.	No. of determinations.	Deviation from average.	Albumin.	Globulin.	No. of determinations.	Deviation from average.	Ratio, globulin: albumin.
	gm. per 100 cc.		*per cent*	*gm. per 100 cc.*	*gm. per 100 cc.*		*per cent*	
1	7.12	4	+0.2, −0.5 +0.9, −0.5	4.80	2.33	3	−2, +0.8, +1.2	1:2.06
2	9.26	4	+0.1, +1.4 −0.3, −1.1	6.43	2.82	3	−0.4, +0.7 +0.1	1:2.27
3	8.07	4	−1.5, −0.2 +0.5, +1.2	5.40	2.67	4	−0.3, +0.2 +0.1, −0.2	1:2.02
4	7.65	4	+0.4, −1.9 +1.5, +0.1	5.13	2.51	4	+0.2, −2.3 +1.7, +0.5	1:2.04
5	7.78	4	−1.5, +2.6 −2.7, +1.6	5.22	2.56	4	+2.5, −0.8 −2, +0.5	1:2.04

The blood was taken from one person on different dates (December 27, 1927; January 10, January 30, February 8, February 22, 1928). It was not taken in the fasting state, but uniformity of conditions was maintained as much as possible. On January 10 (Sample 2) the blood became hemolyzed.

For determination of total nitrogen we have used a dilution corresponding to 0.1 cc. of serum. For determination of albumin plus non-protein nitrogen we have used a dilution corresponding to $\frac{1}{4}$ cc. of serum. For determination of non-protein nitrogen we have used a dilution corresponding to 0.5 cc. of serum.

be cooled sufficiently. The excess acid is titrated with 0.05 N sodium hydroxide, with methyl red and methylene blue as indicators.

A micro burette of 5 cc. capacity, having subdivisions of 0.02 cc., is used.

The method gives satisfactory results on ammonium sulfate and urea, and also on serum, as shown in Tables I and II. It is also shown that for the same person the ratio globulin:albumin remains constant for a period of about 2 months, if the technique for obtaining blood is correct.

BIBLIOGRAPHY.

1. Cullen, G. E., and Van Slyke, D. D., *J. Biol. Chem.*, 1920, xli, 587.
2. Petschacher, L., Berger, W., and Schretter, G., *Z. ges. exp. Med.*, 1926, l, 449.
3. Fuchs, H., *Biochem. Z.*, 1926, clxxvi, 32.

CLINICAL CALORIMETRY.

XLII. A COMPARISON OF THE EFFECT OF GLUCOSE AND DIHYDROXYACETONE ON METABOLISM.

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INTRODUCTION.

The study of the intermediary metabolism of glucose has recently occupied the attention of a number of investigators who have approached the subject by using the triose dihydroxyacetone, $CH_2OH \cdot CO \cdot CH_2OH$. Rabinowitch (1) and Mason (2) have reviewed the earlier work with dihydroxyacetone which was limited on account of difficulties in its preparation. They noted in the course of their own investigations that blood sugar curves in normal subjects, following dihydroxyacetone administration, were only slightly elevated in comparison with those following an equal amount of glucose. Mason (2) found in normal men a more rapid and greater elevation of non-protein respiratory quotient, carbohydrate combustion, and total heat production after giving dihydroxyacetone than after giving glucose. In diabetic patients, Rabinowitch (1, 3) and Kermack, Lambie, and Slater (4) report improvement following the ingestion of dihydroxyacetone on the basis of a falling blood sugar level, reduction in the severity of ketosis, and improvement in general clinical appearance. Mason (5) reported in diabetic subjects a greater rise in the non-protein respiratory quotient, carbohydrate combustion, and heat production after dihydroxyacetone than after glucose. He noted at the same time that the rise in blood sugar curves was less marked and the return to fasting level was quicker after dihydroxyacetone. Himwich, Rose, and Maler (6) found a more rapid rise in the respiratory quotient of dogs after injecting dihydroxyacetone subcutaneously than after injecting the same amount of glucose.

Campbell and his collaborators studied the action of dihydroxyacetone

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in depancreatized dogs. They (7) noted no preferential utilization of dihydroxyacetone in these animals when it was compared with levulose, inulin, and glycerol. Campbell and Markowitz (8) reported increased oxygen consumption without elevation of the respiratory quotient, which was at the fat level. They discovered dihydroxyacetone present in the blood and noted a rise in the actual blood sugar which was coincident with the disappearance of dihydroxyacetone from the blood. They also found "extra glucose" in the urine equivalent to the amount of dihydroxyacetone ingested. They concluded that dihydroxyacetone was converted into glucose in the animal body, that insulin was not required for this conversion, and that it could not be metabolized directly by depancreatized animals. Markowitz and Campbell (9) observing animals after the liver, stomach, and intestines had been removed, reported that dihydroxyacetone injected intravenously was not converted into glucose but that it could be recovered from tissues and blood in sufficient quantities to account for the amounts given. They concluded that the conversion of dihydroxyacetone to glucose occurred in the liver. Campbell and Hepburn (10), Hewitt and Reeves (11), and Kermack, Lambie, and Slater (4) all found that dihydroxyacetone would relieve insulin hypoglycemia at approximately the same rate as glucose.

The studies with dihydroxyacetone presented in this paper were carried out in the calorimeter of the Russell Sage Institute in order to obtain the results of long period observations which can be made with this apparatus. Determinations were made on four normal and two diabetic men. In order to compare the metabolism of long periods as obtained in the calorimeter with the so called short period observation, five of these six subjects were studied with the Tissot apparatus thus giving in addition an opportunity for obtaining frequent samples of blood and urine.

Methods.

The respiration calorimeter of the Russell Sage Institute of Pathology was used to obtain the metabolic data for the long period observations. It has previously been described in Paper II of this series (12). No change in procedure was used except that the temperature of the air in the calorimeter was kept between 24.0° and 25.0°. One normal and two diabetic subjects were studied in the metabolism ward in connection with the calorimeter (13). The other three normal subjects were physicians who came to the laboratory without breakfast and rested for 1 to 2 hours before actual observations were started. The observations were

3 hours in length. Each subject was observed at least three times, once in basal conditions, once with glucose, and once with dihydroxyacetone. A thermos bottle with a small syphon was used so that the subject could easily take the solution, when inside of the calorimeter, after the preliminary period was completed. In basal observations the subjects were given 200 cc. of water, ½ grain of saccharin, and 0.5 gm. of citric acid making a palatable drink which was used as a control. In the other observations the saccharin was replaced by 50 gm. of glucose or by 50 gm. of dihydroxyacetone. The solution was placed in the thermos bottle at 38° so that it could be taken at approximately body temperature 30 to 45 minutes later when the observation was actually started. The urine was collected at the end of the observation. Nitrogen was determined by the Kjeldahl technique and sugar, if present, by Benedict's method (14).

In making the short period observations, the Tissot apparatus, a nose clip, mouthpiece, and 2-way valve, the Soderstrom model of the Lovên diaphragm valve (15), were used for the collection of expired air. The expired air was analyzed by a modified Henderson-Haldane gas analysis apparatus. The procedure with each subject was uniform and consisted of a basal period after at least 30 minutes rest, the ingestion of 50 gm. of either glucose or dihydroxyacetone, and observations taken at the end of 30 minutes, 1 hour, 2 hours, and 3 hours. The expired air was collected during the last 6 minutes of each period, a sample of blood taken at the end of each period, and a sample of urine voided at the end of each hour. Each sample of blood was tested for blood sugar by the method of Folin and Wu (16), for dihydroxyacetone by the method of Campbell (17), and in some observations for carbon dioxide-combining power by the method of Van Slyke and Cullen (18). Each sample of urine was tested for total nitrogen by the Kjeldahl method, for sugar, if present, by Benedict's method (14), and for dihydroxyacetone by the method of one of the authors (19).

As a control on the experimental data frequent alcohol checks were made on the calorimeter. The alcohol solution employed for these checks contained 92.07 per cent of alcohol by weight. The amount used per hour was between 9.7 and 11.5 cc. With calculations based on the chemical composition of the alcohol and

TABLE I.

Alcohol Checks for 1926–27.

Date.	Hour.	Error, per cent.				R. Q., theory 0.667.
		Heat.	O_2	CO_2	H_2O	
1926						
Oct. 20	1	+1.3	+0.7	+0.2	+17.0	0.663
	2	+0.3	−0.8	−1.5	+10.3	0.662
	3	+0.8	−1.9	−1.4	+10.3	0.671
Average		+1.0	−0.7	−0.9	+12.6	0.665
Oct. 28	1	+0.3	−1.6	−0.3	+6.0	0.676
	2	+0.9	−1.8	−1.0	+5.5	0.672
	3	+2.9	−1.3	+0.8	+6.5	0.681
Average		+1.4	−1.5	−0.1	+6.0	0.676
Nov. 4	1	−0.4	−2.6	−0.4	+3.8	0.681
	2	+0.8	−0.6	−0.5	+4.2	0.668
	3	−0.8	−1.8	−0.5	+3.0	0.675
Average		−0.1	−1.7	−0.5	+3.7	0.675
1927						
Jan. 4	1	−0.5	+0.2	−0.8	+4.2	0.660
	2	−0.8	−1.7	−0.4	+4.5	0.676
	3	±0.0	−1.6	−0.3	+2.2	0.675
Average		−0.4	−1.0	−0.5	+3.7	0.670
Feb. 3	1	−4.8	−3.8	−2.1	+2.2	0.679
	2	−1.3	−1.0	−1.8	+4.2	0.661
	3	−1.2	−2.3	−1.1	+1.9	0.674
Average		−2.5	−2.4	−1.7	+2.8	0.671
Feb. 15	1	−1.2	−3.8	−1.8	+3.4	0.681
	2	−0.5	−0.3	+0.4	+4.3	0.672
	3	−1.0	−0.7	−2.3	+1.6	0.656
Average		−0.9	−1.6	−1.2	+3.1	0.669
Mar. 1	1	−1.9	−2.8	−1.9	+3.4	0.673
	2	−1.0	−3.0	−3.4	+2.7	0.664
	3	−1.3	−2.4	−1.5	+3.0	0.673
Average		−1.4	−2.7	−2.3	+3.0	0.670
Mar. 17	1	+0.5	−1.4	−0.3	+4.2	0.674
	2	+1.2	−3.7	−0.6	+4.4	0.690
	3	+1.8	±0.0	−1.3	+5.8	0.658
Average		+1.2	−1.7	−0.7	+4.7	0.674

TABLE I—*Concluded.*

Date.	Hour.	Error, per cent.				R. Q., theory 0.667.
		Heat.	O_2	CO_2	H_2O	
1927						
Mar. 29	1	−0.5	−3.9	−1.4	+2.2	0.683
	2	+0.7	−1.1	+1.0	+4.8	0.681
	3	+0.8	−0.5	+1.3	+3.7	0.679
Average		+0.3	−1.9	+0.3	+3.3	0.680
Mar. 31	1	−1.2	−3.1	−0.6	+3.4	0.683
	2	−1.1	−1.3	−1.3	+3.6	0.666
Average		−1.1	−2.2	−1.0	+3.5	0.675
Apr. 5	1	−2.2	−0.2	−1.6	+4.4	0.657
	2	−0.5	−1.7	−1.6	+2.9	0.667
	3	−0.1	−0.6	−1.3	+4.0	0.662
Average		−0.9	−0.8	−1.5	+3.8	0.662
Apr. 28	1	−1.4	−1.0	−0.5	+5.3	0.670
	2	−0.8	+0.2	−1.8	+3.1	0.653
	3	−1.8	−1.0	−1.0	+3.6	0.666
Average		−1.3	−0.6	−1.1	+4.0	0.663
Total average		−0.4	−1.6	−0.9	+4.5	0.671

complete combustion assumed at an air temperature of 24.0°, 10 cc. of this solution give the following theoretical factors: O_2 15.53 gm., CO_2 14.23 gm., H_2O 9.38 gm., and heat 52.73 calories. The results of all the alcohol checks are presented in Table I. The gas analysis apparatus was tested by frequent outdoor air analyses. All chemical determinations were carried out in duplicate. A trained subject in basal condition was studied with the Tissot apparatus and then placed in the calorimeter for further basal observations.

PROTOCOLS.

Subject 1, E. M., Normal.—A laborer, age 39, Russian, was admitted to Bellevue Hospital January 15, 1927, complaining of weakness and vague pains in the left side of the body. For 10 months, since a slight accident, he noticed discomfort throughout the left side of the body and more pain in the left chest.

Physical examination showed an apathetic complaining man with some hyperesthesia over the left chest but otherwise there were no abnormal findings.

Laboratory tests revealed normal blood and urine, Wassermann test negative, normal electrocardiogram, and x-ray of heart and lungs normal.

During his stay in the hospital there were periods when he seemed somewhat depressed but no psychosis developed. He was studied as a normal control with four calorimeter and two Tissot observations. In the first

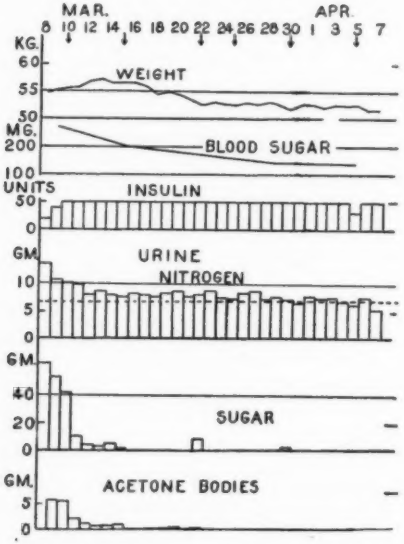

CHART I. Subject C. W.; laboratory findings shown graphically. The broken line signifies the nitrogen in the food; the arrows, the points at which the metabolism was observed.

calorimeter observation his temperature by rectum was 38.5° so it has been excluded from the comparisons.

Subject 2, A. B., Normal.—A physician, age 24, Argentinian, who was in good health. He was studied with four calorimeter and two Tissot observations.

Subject 3, H. J. D., Normal.—A physician, age 29, American, who was in good health. He was studied with three calorimeter observations.

Subject 4, W. S. M., Normal.—A physician, age 31, American, who was in good health. He was studied with three calorimeter and two Tissot observations.

Subject 5, C. W., Diabetes Mellitus.—A baker, age 29, Austrian, was admitted to Bellevue Hospital March 5, 1927, complaining of polydipsia, polyuria, and loss of weight. His history of diabetes dated back 1 year during which he had been on a moderately restricted diet at home. The symptoms noted had appeared during the past month.

Physical examination showed a thin young man in no pain, with some poor teeth, moderately large tonsils, scars from burns on left arm, and otherwise normal.

He had a normal temperature, pulse, and respiration. The remaining laboratory data with daily weights and insulin used are presented in Chart I. He received a diet of 1800 calories made up of protein 42 gm., fat 148

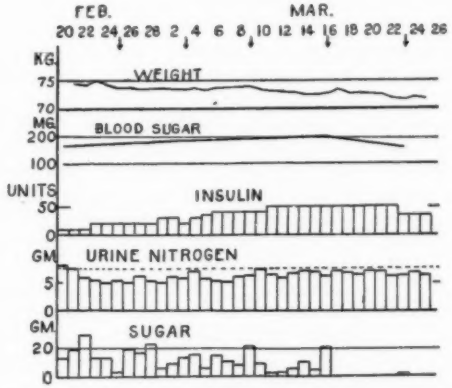

CHART II. Subject J. M.; laboratory findings shown graphically. The broken line signifies the nitrogen in the food; the arrows, the points at which the metabolism was observed.

gm., and carbohydrate 62 gm. until March 27, when it was increased to 2000 calories with protein 42 gm., fat 165 gm., and carbohydrate 71 gm. His condition improved rapidly with regulation of diet and insulin. He showed a mild ketosis on admission. Four calorimeter and two Tissot observations were made. The first calorimeter observation was made with dihydroxyacetone while he was still in a mild ketosis. The other five were made after his condition had improved.

Subject 6, J. M., Diabetes Mellitus.—A salesman, age 43, American, was admitted to the Neurological service of Bellevue Hospital February 7, 1927, complaining of loss of vision and pain in the legs. He had had severe burns from gasoline, in 1911, and gonorrhea, in 1918. His disturbance of vision was first noted 6 months ago and since then it has progressed so that he can only recognize large objects and has difficulty in finding his way

about the city. For a year he has noted sharp pains in both legs and weakness of the knees when climbing stairs. There has been some pain in the back, mostly in the shoulder and upper lumbar regions. He has lost 100 pounds in the past 2 years.

Physical examination revealed a moderately obese man with loose dry skin. Pupils reacted to light and accommodation. Fundi and external muscles of eye were normal. Heart sounds were distant. Blood vessels were palpable but not beaded, lower extremities were cold. Knee jerks were present but very sluggish.

Laboratory findings showed temperature 36–38°, pulse 60 to 80, respiration 16 to 24, blood and spinal fluid Wassermann tests negative, and urine with 1 to 2 per cent sugar and a trace of acetone.

A diagnosis of diabetes was made and the patient transferred to the Medical service. His case proved to be somewhat resistant to treatment and symptoms were very slow to improve. While under observation a severe pain developed in the right side of his face and x-ray revealed a cloudy sinus. It was drained and foul pus obtained followed by relief of pain and general improvement in his condition. He received a diet of 2000 calories made up of protein 48 gm., fat 164 gm., and carbohydrate 68 gm. Chart II contains the data of weight, insulin units, and blood and urine examinations during the period of actual observations. He was studied with three calorimeter and four Tissot observations all of which, except the final basal series with the Tissot apparatus, were made during the period before his sinus showed the signs of infection.

Subject 7, E. F. D. B., Normal.—A physician, age 44, American, who was in good health and whose metabolism has been studied frequently for the past 14 years (20). His basal metabolism was determined by both Tissot apparatus and calorimeter on the same day. No observations with either glucose or dihydroxyacetone were made.

Comment on Experimental Data.

Table II contains the complete data of all calorimeter observations. The 1st hour, recorded in the time column, is the end of the preliminary period which represents the actual start of the observation. All periods were 1 hour in length except as noted in the table in the case of A.B. and W.S.M., where on two occasions the period was 2 hours in length. The marked increase in oxygen used by W.S.M. in the observation with dihydroxyacetone may partly be due to a mild headache causing some extra movements during the early part of the experiment. The per cent of calories per hour from each type of food metabolized is based on the amount of nitrogen in the urine, which multiplied by 26.51 gives the calories from protein and on the table of Zuntz and Schumburg

as modified by Lusk (21) which gives the partition of the non-protein calories between fat and carbohydrate. The carbohydrate in gm. represents the amount of carbohydrate metabolized per hour on this basis.

Table III presents the complete data of the Tissot observations. The figures represent data for 1 hour periods as calculated from that obtained in a 6 minute observation. We can give no explanation for the marked elevation of carbon dioxide in the last period of the observation of C. W. with glucose. The columns under blood and urine headed "R. S." represent the total amount of M-substances of which the composition is not known and which will reduce the phosphomolybdate solution used in their determination. To determine the amounts of dihydroxyacetone present, the values obtained for basal blood and urine are taken as blanks and deducted from the values obtained in the other samples. The slight differences found were not tabulated as dihydroxyacetone in blood because they were considered to be within the range of error of the method used.

Charts III and IV are graphic presentations of carbon dioxide elimination, oxygen consumption, respiratory quotient, and heat production of the subjects E.M. and C.W.

DISCUSSION.

Dihydroxyacetone, taken by mouth, caused an increased output of carbon dioxide during the 1st hour as determined by both Tissot and calorimeter methods. It reached a very high level at the end of 30 minutes as noted by the Tissot studies. There was also an increased oxygen consumption which was most marked within the 1st hour. A rapid rise of the respiratory quotient occurred during that same period. The maximum quotient obtained in the calorimeter was 0.99 and with the Tissot method 1.09. With two subjects in the calorimeter the highest quotient occurred during the 2nd hour. An elevation of the respiratory quotient is produced either by an increased carbon dioxide elimination or by a decreased oxygen absorption. The increase noted after dihydroxyacetone was due to a marked increase in carbon dioxide elimination, as there was no decrease in oxygen absorption.

The cause of the marked increase in carbon dioxide elimination

TABLE II.

Calorimeter Data.

Subject, date (1927), weight, and surface area.	End of period.	CO_2	O_2	R. Q.	H_2O	Urine N per hr.	Non-protein R. Q.	Indirect calorimetry.	Heat eliminated.	Direct calorimetry.	Rectal temperature.	Pulse rate.	Work added.	Per cent calories from: Protein.	Fat.	Carbohydrate.	Carbohydrate per hr.	Remarks.
		gm.	*gm.*		*gm.*	*gm.*		*calories*	*calories*	*calories*	°*C.*		*cm.*				*gm.*	
E. M., Jan. 21,	12.05										38.31							Temperature elevated.
68.4 kg., 1.79	1.05	25.2	23.5	0.78	34.2	0.49	0.77	77.6	75.4	81.9	38.43			17	64	19	3.7	
sq. m.	2.05	25.6	23.0	0.81	33.7	0.49	0.81	76.5	77.4	78.2	38.45			17	52	31	5.7	
(Normal.)	3.05	25.7	24.2	0.77	34.5	0.49	0.77	79.7	80.0	81.2	38.48			17	64	19	3.8	
E. M., Jan. 26,	11.48										37.02							Basal.
68.0 kg., 1.78	12.48	21.0	17.8	0.88	26.0	0.24	0.87	60.4	63.9	64.1	37.03		28	10	38	52	7.6	
sq. m.	1.48	21.8	19.3	0.82	27.2	0.24	0.83	64.6	65.4	67.9	37.08			10	50	40	6.2	
	2.48	21.9	19.6	0.81	28.0	0.24	0.81	65.7	66.1	66.9	37.10			10	57	33	5.4	
E. M., Feb. 4, 67.8	11.47										37.18							Glucose 50 gm. at 11.03 a.m.
kg., 1.78 sq. m.	12.47	23.2	18.6	0.91	33.1	0.33	0.93	63.6	67.7	59.5	37.04		4	14	20	66	10.2	
	1.47	22.4	18.1	0.90	30.9	0.33	0.92	61.7	66.2	67.6	37.07		10	14	23	63	9.5	
	2.47	22.2	17.2	0.94	29.9	0.33	0.97	59.3	65.2	60.4	36.99		13	15	8	77	11.1	
E. M., Jan. 28,	11.42										37.11							Dihydroxyacetone 50 gm. at 11.04 a.m.
67.9 kg., 1.78	12.42	23.6	18.2	0.95	27.7	0.30	0.97	62.7	66.0	61.7	37.04		7	12	9	79	12.1	
sq. m.	1.42	22.8	18.5	0.90	28.2	0.30	0.91	63.1	68.3	68.5	37.05		4	12	25	63	9.5	
	2.42	21.5	19.0	0.82	28.2	0.30	0.82	63.7	69.2	62.1	36.93		6	12	53	35	5.4	
A. B., Feb. 2, 56.3	11.37										37.22	66						Basal.
kg., 1.61 sq. m.	12.37										37.12	72	26					

	2.42	21.5	19.0	0.82	28.2	0.30	0.82	63.7	69.2	62.1	36.93		6	12	53	35	5.4	
A. B., Feb. 2, 56.3 kg., 1.61 sq. m. (Normal.)	11.37										37.22	66						Basal.
	12.37										37.12	72	26					
	1.37	41.1*	33.1	0.90	56.5	0.69	0.96	111.5	131.5	125.7	37.11	68	12	33	9	58	8.0	
	2.37	20.7	16.9	0.89	26.3	0.69	0.94	56.8	63.6	67.0	37.19	68	8	32	13	55	7.6	
A. B., Mar. 4, 57.2 kg., 1.62 sq. m.	11.30										36.96							Saccharin ½ gr. at 11.33 a.m.
	12.30	20.5	16.8	0.89	21.7	0.62	0.93	56.5	57.5	63.0	37.08	68	10	29	16	55	7.6	
	1.30	19.8	16.9	0.85	22.3	0.62	0.87	56.5	59.6	57.5	37.04	65	10	29	30	41	5.6	
	2.30	20.6	17.3	0.86	22.7	0.62	0.89	58.0	57.0	59.6	37.10	64		28	26	46	6.5	
A. B., Feb. 23, 56.9 kg., 1.61 sq. m.	12.00										37.06							Glucose 50 gm. at 12.03 p.m.
	1.00	23.2	18.0	0.94	28.4	0.59	0.99	61.4	62.9	64.5	37.10	72	12	25	2	73	10.9	
	2.00	23.3	17.6	0.96	27.2	0.59	1.02	60.5	63.3	61.1	37.06	66	10	26	0	74	11.0	
	3.00	22.3	17.1	0.95	25.9	0.59	1.01	58.4	60.8	59.6	37.04	68	12	27	0	73	10.5	
A. B., Feb. 18, 56.5 kg., 1.61 sq. m.	11.42										37.04							Dihydroxyacetone 50 gm. at 11.48 a.m.
	12.42	23.7	17.4	0.99	31.0	0.78	1.10	59.4	60.7	64.1	37.12	68	24	35	0	65	9.3	
	1.42	20.4	16.0	0.93	28.2	0.78	1.02	53.8	60.1	62.1	37.17	62	4	38	0	62	8.1	
	2.42	24.4	18.6	0.95	28.9	0.78	1.04	63.4	61.0	65.4	37.27	68		33	0	67	10.4	
H. J. D., Mar. 11, 85.5 kg., 2.00 sq. m. (Normal.)	11.21										37.29							Saccharin ½ gr. at 11.25 a.m.
	12.21	23.8	20.5	0.85	30.1	0.40	0.86	68.8	73.2	60.2	37.11		4	15	40	45	7.6	
	1.21	23.5	20.1	0.85	27.2	0.40	0.86	67.8	72.4	59.3	36.93		3	16	38	46	7.6	
	2.21	22.4	20.2	0.80	26.2	0.40	0.80	67.3	73.1	66.4	36.84		4	16	57	27	4.5	
H. J. D., Apr. 12, 86.2 kg., 2.00 sq. m.	11.35										37.09							Glucose 50 gm. at 11.39 a.m.
	12.35	24.9	21.6	0.84	27.4	0.38†	0.84	72.5	69.9	58.9	36.94	56	6	14	45	41	7.2	
	1.35	24.9	20.4	0.89	26.6	0.38	0.90	69.3	76.6	59.8	36.71	58	3	15	27	58	9.8	
	2.35	28.0	21.2	0.96	28.2	0.38	0.99	73.4	73.0	79.2	36.80	58	16	14	3	83	14.9	

* 2 hour period.

† Average of two other determinations.

TABLE II—*Concluded.*

Subject, date (1927), weight, and surface area.	End of period.	CO_2	O_2	R. Q.	H_2O	Urine N per hr.	Non-protein R. Q.	Indirect calorimetry.	Heat eliminated.	Direct calorimetry.	Rectal temperature.	Pulse rate.	Work adder.	Per cent calories from: Protein.	Fat.	Carbohydrate.	Carbohydrate per hr.	Remarks.
		gm.	*gm.*		*gm.*	*gm.*		*calories*	*calories*	*calories*	°*C.*		*cm.*				*gm.*	
H. J. D., Mar. 18,	11.42										36.97							Dihydroxyacetone 50 gm. at
85.3 kg., 2.00	12.42	27.1	22.0	0.90	34.7	0.36	0.91	75.1	74.5	59.3	36.76		5	13	25	62	11.4	
sq. m.	1.42	28.8	21.3	0.98	32.0	0.36	1.01	74.3	76.5	76.9	36.77		3	13	0	87	15.9	11.47 a.m.
	2.42	22.1	19.3	0.83	29.6	0.36	0.84	64.8	74.0	58.2	36.55		5	15	45	40	6.3	
W. S. M., Mar. 24,	11.52										37.02							Saccharin ½ gr. at
78.0 kg., 1.93	12.52	24.1	21.5	0.82	31.0	0.43	0.82	71.8	73.0	67.5	36.94	76	17	16	50	34	5.9	11.56 a.m.
sq. m.	1.52				31.0				72.1	60.8	36.77	72	16					
(Normal.)	2.52	50.8*	44.0	0.84	34.1	0.43	0.85	147.8	76.4	85.8	36.92	70	12	16	41	43	7.8	
W. S. M., Apr. 19,	11.38										37.19							Glucose 50 gm. at
78.8 kg., 1.93	12.38	26.3	23.0	0.83	39.9	0.45	0.84	76.9	81.2	71.0	37.04		14	16	44	40	7.6	11.42 a.m.
sq. m.	1.38	26.4	21.8	0.88	37.2	0.45	0.90	73.8	81.2	80.2	37.03		14	16	27	57	10.3	
	2.38	27.2	21.4	0.92	38.2	0.45	0.95	73.4	80.7	71.8	36.90		17	16	13	71	12.7	
W. S. M., Apr. 14,	11.32										36.96	80						Dihydroxyacetone 50 gm. at
79.1 kg., 1.93	12.32	30.3	25.7	0.86	38.5	0.64	0.87	86.4	83.3	79.7	36.91	76	21	20	35	45	9.5	
sq. m.	1.32	31.1	24.9	0.91	39.6	0.64	0.94	84.7	85.3	79.7	36.83	76	19	20	15	65	13.4	11.36 a.m. Mild
	2.32	25.3	21.6	0.85	36.8	0.64	0.87	72.3	79.7	78.6	36.82	72	14	24	32	44	7.8	headache.
C. W., Mar. 15,	11.57										36.59							Saccharin ½ gr.
56.3 kg., 1.63	12.57	18.2	16.9	0.78	27.6	0.25	0.78	56.0	61.0	52.9	36.42		4	11	66	23	3.2	at 12.00 n.
sq. m.	1.57	17.2	16.1	0.78	24.1	0.25	0.78	53.3	58.4	55.3	36.36		2	12	66	22	2.9	
(Diabetic.)	2.57	17.5	16.6	0.77	23.4	0.25	0.76	54.9	56.5	47.5	36.17			12	71	17	2.2	
C. W., Mar. 22,	12.15										36.42							Glucose 50 gm. at

56.3 kg., 1.63 sq. m. (Diabetic.)	12.57	18.2	16.9	0.78	27.6	0.25	0.78	56.0	61.0	52.9	36.42	4	11	66	23	3.2	at 12.00 n.
	1.57	17.2	16.1	0.78	24.1	0.25	0.78	53.3	58.4	55.3	36.36	2	12	66	22	2.9	
	2.57	17.5	16.6	0.77	23.4	0.25	0.76	54.9	56.5	47.5	36.17		12	71	17	2.2	
C. W., Mar. 22, 53.5 kg., 1.60 sq. m.	12.15										36.42						Glucose 50 gm. at 12.18 p.m.
	1.15	17.3	15.8	0.79	23.6	0.34	0.79	52.5	58.9	51.6	36.26	16	17	58	25	3.2	
	2.15	17.1	15.8	0.79	22.5	0.34	0.78	52.4	55.9	52.2	36.18	13	17	61	22	2.8	
	3.15	17.5	15.6	0.82	21.4	0.34	0.82	51.9	55.1	48.8	36.04		17	50	33	4.1	
C. W., Mar. 10, 54.9 kg., 1.61 sq. m.	11.42										36.34						Dihydroxyacetone 50 gm. at 11.45 a.m. Patient in mild acidosis.
	12.42	23.8	21.2	0.82	23.7	0.46	0.82	70.8	62.9	60.0	36.28	7	17	50	33	5.7	
	1.42	19.4	19.9	0.71	22.8	0.46	0.69	64.6	62.1	60.1	36.24	3	19	81	0	0	
	2.42	18.3	18.0	0.74	22.5	0.46	0.72	58.6	64.0	58.8	36.13	7	21	75	4	0.6	
C. W., Mar. 25, 52.5 kg., 1.59 sq. m.	11.51										36.51						Dihydroxyacetone 50 gm. at 11.53 a.m. No acidosis.
	12.51	21.9	16.7	0.95	23.8	0.27	0.98	57.9	53.6	48.6	36.40	10	12	5	83	11.5	
	1.51	18.5	17.2	0.78	23.1	0.27	0.78	57.0	58.9	52.6	36.26	8	12	65	23	3.2	
	2.51	17.0	16.5	0.75	21.6	0.27	0.74	54.2	53.2	48.6	36.16	9	13	77	10	1.3	
J. M., Feb. 25, 74.1 kg., 1.86 sq. m. (Diabetic.)	11.51										36.91						Saccharin ½ gr. at 11.57 a.m.
	12.51	20.3	19.2	0.77	26.4	0.21	0.77	63.5	65.2	61.2	36.85		9	71	20	3.2	
	1.51	19.0	18.0	0.77	24.9	0.21	0.76	59.5	65.9	68.1	36.89		9	74	17	2.4	
	2.51	19.1	18.1	0.77	24.1	0.21	0.76	60.1	65.7	64.3	36.87		9	73	18	2.7	
J. M., Mar. 9, 72.2 kg., 1.84 sq. m.	11.32										37.01						Glucose 50 gm. at 11.35 a.m.
	12.32	21.6	20.4	0.77	27.6	0.33	0.76	67.5	73.6	70.3	36.96	17	13	71	16	2.7	
	1.32	20.9	20.1	0.76	28.3	0.33	0.75	66.1	73.6	63.1	36.79	17	13	73	14	2.2	
	2.32	19.9	19.1	0.76	26.6	0.33	0.75	62.9	70.1	65.6	36.72	7	14	72	14	2.2	
J. M., Mar. 3, 72.7 kg., 1.84 sq. m.	12.35										36.82						Dihydroxyacetone 50 gm. at 12.39 p.m.
	1.35	21.8	20.0	0.79	23.5	0.25	0.79	66.6	68.5	68.8	36.83	28	10	63	27	4.4	
	2.35	21.0	19.4	0.79	23.4	0.25	0.79	64.5	69.4	66.8	36.79	13	10	64	26	4.1	
	3.35	22.0	20.2	0.79	24.1	0.25	0.79	67.2	66.5	62.0	36.72	9	10	63	27	4.4	

TABLE III.

Tissot Data.

Subject, date (1927), and surface area.	Time.	CO_2	O_2	R.Q.	Urine N per hr.	Non-protein R.Q.	Calories per hr.	Pulse rate.	Respiratory rate.	Per cent calories from:			Carbohydrate per hr.	Blood.			Urine.		Remarks.
										Protein.	Fat.	Carbohydrate.		Sugar.	R.S.*	CO_2	Sugar.	R.S.*	
	min.	*gm.*	*gm.*		*gm.*								*gm.*	*mg. per 100 cc.*	*mg. per 100 cc.*	*per cent*	*gm. per hr.*	*mg. per hr.*	
E. M., Feb. 1,	Basal.	20.1	16.8	0.87	0.46	0.89	56.6	58	16	22	28	50	7.0	87	13		0	29	Normal; glu-
1.78 sq. m.	30	22.4	19.7	0.83	0.49	0.83	65.7	60	13	20	45	35	5.6	109	15		0	36	cose 50 gm.
	60	25.2	20.0	0.92	0.49	0.95	68.3	58	14	19	13	68	11.4	141	14		0	36	
	120	22.1	18.8	0.86	0.43	0.87	63.3	60	13	18	35	47	7.3	133	12		0	29	
	180	21.7	17.9	0.89	0.43	0.91	60.3	58	18	19	23	58	8.5	95	11		0	29	
E. M., Feb. 9.	Basal.	21.7	19.5	0.81	0.41	0.81	65.0	61	16	17	52	31	4.9	91	15		0	28	Dihydroxyace-
	30	31.1	22.7	1.00	0.50	1.04	79.0	65	17	17	0	83	16.1	97	17		0	70	tone 50 gm.
	60	23.3	21.2	0.80	0.50	0.80	70.3	68	16	19	54	27	4.6	80	17		0	70	
	120	24.0	19.8	0.88	0.48	0.90	67.1	60	17	19	26	55	9.0	89	18		0	95	
	180	22.8	20.4	0.81	0.37	0.82	68.0	62	16	14	51	35	5.7	89	15		0	87	
A. B., Apr. 29,	Basal.	18.8	17.4	0.79	0.35	0.78	57.6	64	12	16	62	22	3.0	93	11		0	10	Normal; glu-
1.61 sq. m.	30	21.2	18.8	0.82	0.73	0.83	62.2	68	14	31	39	30	4.6	130	11		0	6	cose 50 gm.
	60	23.7	19.4	0.89	0.73	0.93	65.2	66	12	30	16	54	8.7	133	11		0	6	
	120	20.8	17.2	0.88	0.56	0.90	57.9	66	12	26	24	50	7.1	95	12		0	5	
	180	19.8	17.1	0.84	0.56	0.86	57.2	68	12	26	34	40	5.6	78	11		0	5	
A. B., Apr. 22.	Basal.	19.3	17.1	0.82	0.23	0.82	57.3	66	12	11	54	35	5.0	93	10	59	0	7	Dihydroxyace-
																			tone 50 gm.

	180	19.8	17.1	0.84	0.56	0.86	57.2	68	12	26	34	40	5.6	78	11	0	5	
A. B., Apr. 22.	Basal.	19.3	17.1	0.82	0.23	0.82	57.3	66	12	11	54	35	5.0	93	10	59	0	7	Dihydroxyacetone 50 gm.
	30	29.1	19.7	1.07	0.53	1.15	69.7	62	16	20	0	80	13.4	98	10	60	0	19	
	60	21.7	19.3	0.82	0.53	0.82	64.1	70	14	22	47	31	4.9	63	10	60	0	19	
	120	21.3	17.1	0.90	0.48	0.94	58.1	68	13	22	15	63	8.9	107	10	64	0	41	
	180	19.6	18.2	0.78	0.58	0.78	59.7	68	14	26	55	19	2.8	99	10	63	0	45	
W. S. M., Jan. 27, 1.93 sq. m.	Basal.	26.7	24.7	0.79	0.54	0.78	81.5	68	16	18	61	21	4.3	95	14		0	20	Normal; glucose 50 gm.
	30	29.9	27.0	0.81	0.58	0.81	89.8	64	15	17	52	31	6.7	156	14		0	30	
	60	29.1	26.0	0.82	0.58	0.82	86.5	70	17	18	49	33	6.9	145	14		0	30	
	120	24.6	21.6	0.83	0.51	0.84	72.2	70	16	19	43	38	6.7	105	16		0	25	
	180	25.0	22.0	0.83	0.35	0.83	73.6	62	17	13	49	38	6.8	85	18		0	17	
W. S. M., Feb. 11.	Basal.	26.3	22.9	0.84	0.57	0.85	76.5	64	13	20	39	41	7.6	102	12		0	15	Dihydroxyacetone 50 gm.
	30	38.7	25.9	1.09	0.64	1.16	91.8	71	16	20	0	80	17.6	92	11		0	87	
	60	28.1	25.0	0.82	0.64	0.82	83.0	75	16	21	48	31	6.3	66	13		0	87	
	120	26.1	22.5	0.84	0.59	0.85	75.2	62	17	21	39	40	7.3	86	16		0	99	
	180	24.9	22.3	0.81	0.60	0.82	73.9	62	14	21	48	31	5.6	90	16		0	76	
C. W., Mar. 30, 1.61 sq. m.	Basal.	20.9	16.5	0.92	0.13	0.93	56.9	54	9	6	22	72	10.0	111	11	46	0	3	Diabetic; glucose 50 gm.
	30	18.8	16.9	0.81	0.36	0.81	56.3	58	9	17	53	30	4.1	217	11	60	0	17	
	60	20.1	17.3	0.85	0.36	0.86	58.1	58	10	16	39	45	6.3	280	13	56	0	17	
	120	19.2	17.4	0.80	0.34	0.80	57.8	56	10	16	56	28	3.9	333	11	54	1.0	21	
	180	23.7	17.5	0.98	0.40	1.03	60.8	56	11	17	0	83	12.2	274	13	55	1.1	26	
C. W., Apr. 5.	Basal.	17.8	16.7	0.78	0.19	0.77	55.3	56	11	9	70	21	2.8	137	8	61	0	6	Dihydroxyacetone 50 gm.
	30	22.1	17.2	0.94	0.22	0.95	59.2	58	11	10	15	75	10.8	172	9	59	0	17	
	60	19.4	16.8	0.84	0.22	0.84	56.7	62	11	10	48	42	5.9	200	11	53	0	17	
	120	16.3	16.7	0.71	0.35	0.69	54.4	64	11	17	83	0	0	211	10	57	0	84	
	180	17.0	16.7	0.74	0.31	0.73	54.7	62	11	15	79	6	0.9	181	9	62	Trace.	89	

* This column represents the M-substances which are those that will reduce acid phosphomolybdate solutions.

TABLE III—*Concluded.*

Subject, date (1927), and surface area.	Time.	CO_2	O_2	R.Q.	Urine N per hr.	Non-protein R.Q.	Calories per hr.	Pulse rate.	Respiratory rate.	Per cent calories from:			Carbohydrate per hr.	Blood.			Urine.		Remarks.
										Protein.	Fat.	Carbohydrate.		Sugar.	R.S.*	CO_2	Sugar.	R.S.*	
	min.	*gm.*	*gm.*		*gm.*								*gm.*	*mg. per 100 cc.*	*mg. per 100 cc.*	*per cent*	*gm. per hr.*	*mg. per hr.*	
J. M., Mar. 16, 1.84 sq. m.	Basal.	19.5	20.8	0.68	0.22	0.67	68.1	56	17	9	91	0	0	199	12	57	0	16	Diabetic; glucose 50 gm.
	30	21.6	23.5	0.67	0.27	0.66	76.8	67	17	10	90	0	0	282	12	44	2.1	27	
	60	21.8	23.1	0.69	0.27	0.67	75.6	68	17	10	90	0	0	336	12	53	2.1	27	
	120	23.0	22.8	0.73	0.38	0.72	74.6	58	17	13	83	4	0.7	331	12	55	7.3	73	
	180	21.2	21.6	0.71	0.34	0.70	70.6	58	16	13	87	0	0	292	13	56	5.7	57	
J. M., Mar. 23.	Basal.	20.6	20.2	0.74	0.18	0.74	66.6	58	16	7	82	11	1.8	154	9	54	0	14	Dihydroxyacetone 50 gm.
	30	19.5	20.3	0.70	0.20	0.69	66.2	56	17	8	92	0	0	159	10	57	0	26	
	60	20.9	19.2	0.79	0.20	0.79	64.0	62	17	8	64	28	4.3	181	11	54	0	26	
	120	21.7	19.4	0.81	0.25	0.81	64.9	62	16	10	57	33	5.1	224	11	58	0	74	
	180	19.4	21.6	0.66	0.24	0.64	70.4	64	18	9	91	0	0	234	11	59	0.3	108	
J. M., Mar. 28.	Basal.	19.6	20.4	0.70			66.7	54	16										
J. M., May 10.	Basal.	18.4	18.1	0.74	0.27	0.73	59.2	66	20	12	81	7	1.0						All observations basal without removing mouth piece or nose clip.
	25	17.7	17.4	0.74	0.27	0.73	56.9	68	19	13	80	7	1.0						
	45	18.4	18.0	0.75	0.27	0.74	59.0	66	18	12	77	11	1.6						
	65	19.7	19.0	0.76	0.27	0.75	62.4	70	18	12	74	14	2.2						

E. F. D. B., Apr. 7, 1.95 sq. m.	Tissot. Basal. Calorimeter.	22.4	20.7	0.79	0.59	0.78	68.2	50	10	23	57	20	3.3						Comparison of metabolism determined by Tissot method and calorimeter.
	60	22.8	20.7	0.80	0.53	0.80	68.6	50		21	53	26	4.4						
	120	23.4	21.7	0.79	0.53	0.78	71.5	49		20	58	21	3.6						

is not known. It may come from increased oxidation of carbohydrate in the body or from the conversion of carbohydrate

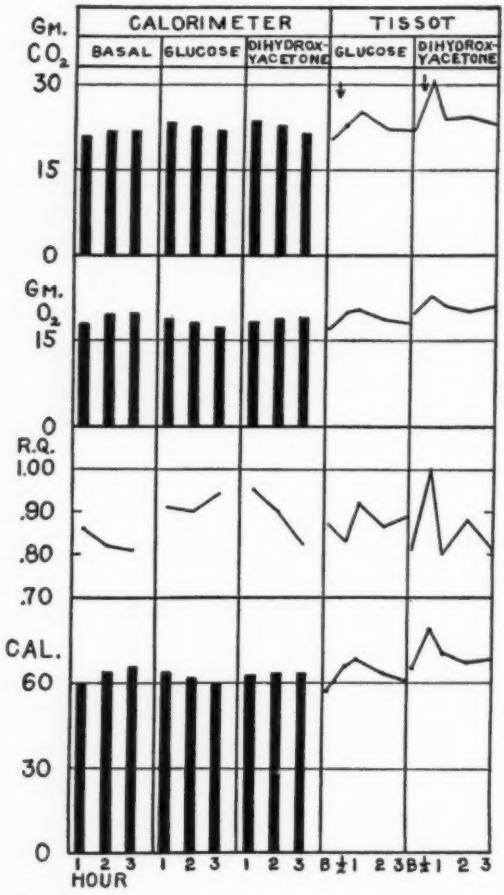

CHART III. Subject E. M., graphic representations of findings in metabolism observations.

to fat. These are the accepted explanations of what happens after the ingestion of carbohydrate when there is an elevation

in the respiratory quotient. Also an increase may occur from overventilation or from the release of carbonic acid when some

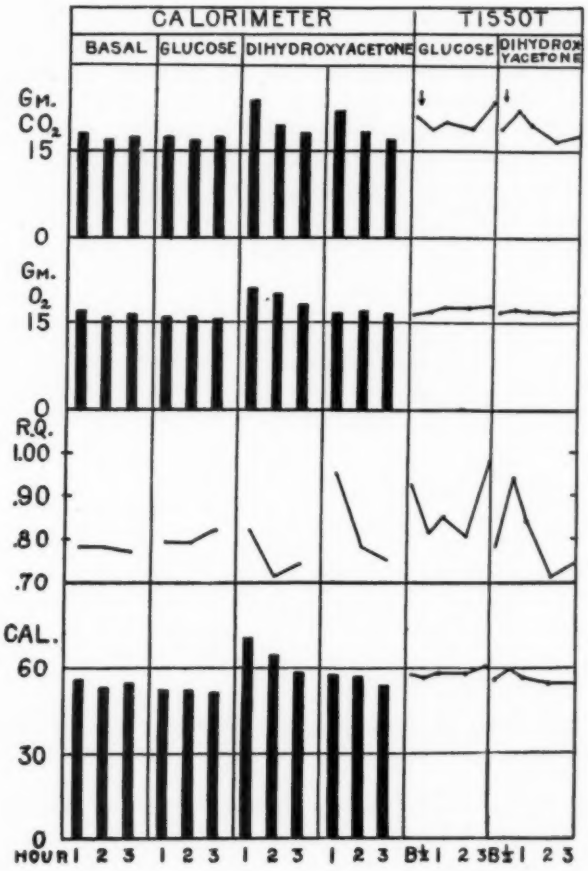

CHART IV. Subject C. W.; graphic representations of findings in metabolism observations.

other acid takes its place in maintaining the acid-base equilibrium of the body. Chart V shows the minute volume of expired air

for each period of the Tissot observations. It was found that the ventilation in normals increased 30 to 50 per cent at the end of 30 minutes after the ingestion of dihydroxyacetone. In one diabetic, C.W., the increase was 18 per cent while in the second, J.M., there was no increase noted. It is interesting that the subject J.M. was the only one who did not show an increased carbon dioxide elimination and an elevated respiratory quotient. The carbon dioxide-combining powers of the blood of three sub-

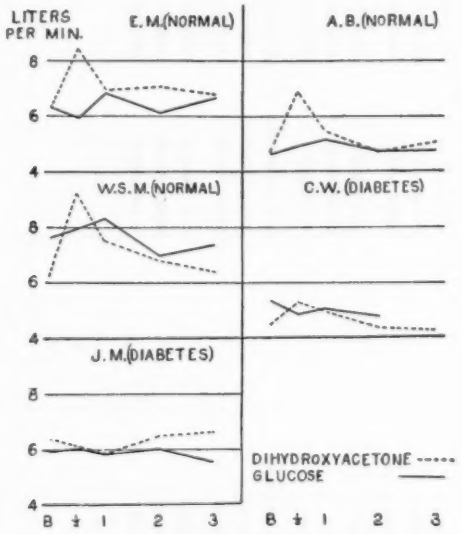

CHART V. Minute volume of expired air for each period of the Tissot observations.

jects were determined and no significant variation from normal was present. Therefore we think that overventilation is one factor in producing the elevated respiratory quotient.

There is an average increased heat production during the 1st hour following the ingestion of 50 gm. of dihydroxyacetone of 8.8 per cent. For the same amount of glucose the increase was 6.9 per cent. When calculated for periods of 3 hours, the elevation is only very slightly greater following dihydroxyacetone than

following glucose. Chart VI shows graphically the average hourly heat production for all observations calculated on the basis of 3 hour experiments. The average percentage increase above basal from 50 gm. of glucose was 3.5 per cent and from the same amount of dihydroxyacetone was 5.0 per cent. Therefore dihydroxyacetone shows almost no variation in heat production from glucose.

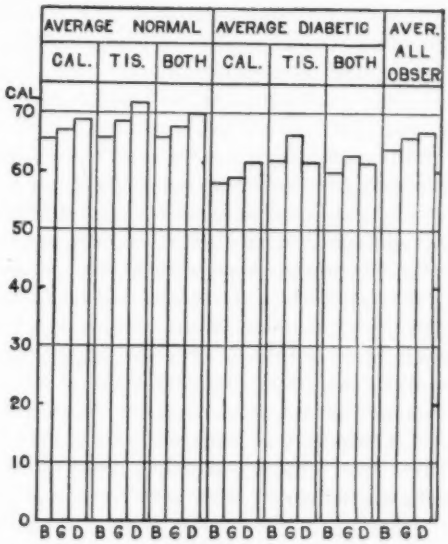

CHART VI. Average hourly heat production for all observations. *B*, basal; *G*, glucose; *D*, dihydroxyacetone.

The observation on C.W. of March 10, 1927, was made while he was in a mild ketosis. He eliminated 5.6 gm. of acetone bodies that day. A respiratory quotient of 0.82 was obtained during the 1st hour with the combustion of 5.7 gm. of carbohydrate. No basal observation was made during this period of ketosis but in other diabetic patients eliminating about the same amount of acetone bodies the basal respiratory quotients average between 0.73 and 0.75. His respiratory quotient for the 3rd

hour was 0.74. This observation suggests that even during a mild ketosis when the body is using only small amounts of carbohydrate it is able to utilize dihydroxyacetone to a greater degree than glucose.

The blood sugar curves in the three normal subjects studied showed a fall in the level at the end of 1 hour after the ingestion of 50 gm. of dihydroxyacetone. This was marked in subjects A.B. and W.S.M. If the dihydroxyacetone is being converted into glucose, this fall in blood sugar is difficult to explain unless one considers either a rapid storage or a rapid oxidation of carbohydrate due to increased production of insulin. In the two diabetics there was an elevation in the blood sugar curves which was less marked than the elevation after glucose. There was no increase in M-substances in the blood after the ingestion of 50 gm. of dihydroxyacetone but there was a slight increase of these substances in the urine in all subjects. The additional amount of M-substances eliminated in the urine was not significant in accounting for the dihydroxyacetone taken as in no subject was more than 0.1 gm. recovered which represented only 0.2 per cent of the 50 gm. ingested. No increase in any subject was found in the M-substances in blood or urine after 50 gm. of glucose were taken.

The abnormally low basal quotients obtained with the Tissot apparatus in one diabetic, J.M., require consideration. They were obtained consistently in repeated tests as may be noted in the data (Table III). There was only a trace of acetone found in the urine during the period of the studies. There was no error detected in technique, in the management of the test, or in the analyses of the gas. The observations were carried out by different observers and the analyses were done by an experienced analyst who obtained consistent checked results both with outside air and with other subjects. The patient's condition was complicated by a sinus infection which was treated during his stay in the hospital. All observations except the one on May 10, 1927, were made before this condition was treated. The observation of May 10, 1927, consisted of a continuous run without removing nose clip or mouthpiece until four separate samples were obtained at approximately 20 minute intervals. These results show as uniform results as can be expected. It was found that these quotients were from 0.03 to 0.08 points lower than those obtained

in the calorimeter. The respiratory quotient curves of this subject show an elevation of 0.05 after glucose and 0.07 after dihydroxyacetone, which are similar to those of other subjects, but the entire curve is at a much lower level. We are unable to explain these quotients.

As an additional check on the two methods of determining metabolism, the basal metabolism of the trained subject E.F.D.B. was obtained with the Tissot apparatus and the calorimeter on the same morning. The data, which are presented in Table III, show a very close uniformity in the results obtained by the two methods. We feel that there may be certain individuals who may give consistent low results as determined by short time (6 to 10 minute) periods. Therefore care should be used in selecting subjects for metabolic investigation. Any conclusions drawn as to the percentage of calories produced by the oxidation of various foodstuffs in a subject like J.M. may show considerable variation when based on data obtained by these two methods.

The way in which dihydroxyacetone is converted and used in the body has not been explained. There is more similarity between the actions of dihydroxyacetone and fructose than between dihydroxyacetone and glucose. Togel, Brezina, and Durig (22), Higgins (23), Benedict and Carpenter (24), Carpenter (25), and Deuel (26) in their studies with sugars found that fructose and sucrose produced a more rapid and greater elevation of the respiratory quotient than glucose. Cori and Cori (27) reported that in rats without insulin 51 per cent of the dihydroxyacetone absorbed is oxidized, 21 per cent deposited as liver glycogen, and 15 per cent as body glycogen. It is interesting to note that both dihydroxyacetone and fructose are ketoses while glucose is an aldehyde. It seems possible that in the body dihydroxyacetone may be converted into an easily oxidizable substance, possibly a form of glucose which has some different characteristics from the ordinary glucose. Perhaps glucose itself before it can be utilized in the body, must be converted into this readily oxidizable form. This oxidizable substance, if it were a form of glucose, could not be utilized in totally diabetic animals and when eliminated it would be detected as glucose. This would also explain the fact that it is more rapidly utilized in normals than is glucose and would account for its partial utilization in diabetics. The

data obtained in this study do not yield any information as to whether or not dihydroxyacetone is an intermediary product in carbohydrate metabolism.

SUMMARY AND CONCLUSIONS.

1. Dihydroxyacetone caused a rapid elevation of the respiratory quotient due to a marked elevation in carbon dioxide elimination. We were unable to determine the source of the excess carbon dioxide eliminated.

2. Dihydroxyacetone resulted in an increased heat production of about 5 per cent in 3 hours which was only slightly in excess of that obtained after glucose. Therefore dihydroxyacetone has approximately the same specific dynamic action as glucose when 50 gm. are taken.

3. No evidence of an increase in the M-substances (those substances, including dihydroxyacetone, which will reduce acid phosphomolybdate solutions) in the blood was obtained after the ingestion of 50 gm. of either dihydroxyacetone or glucose in either normals or diabetics.

4. There was a slight elevation in the M-substances in the urine after dihydroxyacetone. This was not present after glucose.

5. The blood sugar curves in normals showed practically no rise in level after 50 gm. of dihydroxyacetone but on the contrary they showed a fall which was quite marked at the end of 1 hour. In the diabetics there was a rise in the blood sugar level which was less marked than it was after glucose.

6. No positive information was obtained as to whether or not dihydroxyacetone is an intermediary product formed in the metabolism of glucose.

Note.—Since the preparation of this paper, W. R. Campbell reported before the Society for Clinical Investigation at Washington, D. C., April 30, 1928, that, after taking 100 gm. of dihydroxyacetone, there was a fall in the carbon dioxide-combining power of the blood and a marked elevation in blood lactic acid. This may account in part at least for the marked carbon dioxide elimination noted in our observations.

XUM

BIBLIOGRAPHY.

1. Rabinowitch, I. M., Observations on the use of dihydroxyacetone in the treatment of diabetes mellitus, *Canad. Med. Assn. J.*, 1925, xv, 374.
2. Mason, E. H., Dihydroxyacetone studies with respiratory and carbohydrate metabolism in normal men, *J. Clin. Inv.*, 1926, ii, 521.
3. Rabinowitch, I. M., Case of diabetic coma treated with dihydroxyacetone with recovery, *Canad. Med. Assn. J.*, 1925, xv, 520.
4. Kermack, W. O., Lambie, C. G., and Slater, R. H., The utilization of dihydroxyacetone by the animal body, and a method for its determination, *Biochem. J.*, 1926, xx, 486.
5. Mason, E. H., Dihydroxyacetone studies with respiratory and carbohydrate metabolism in diabetes mellitus, *J. Clin. Inv.*, 1926, ii, 533.
6. Himwich, H. E., Rose, M. I., and Maler, M. R., Changes in respiratory quotient produced by the subcutaneous injection of dihydroxyacetone and glucose, *Proc. Soc. Exp. Biol. and Med.*, 1926, xxiv, 38.
7. Campbell, W. R., and Markowitz, J., Preferential utilization of carbohydrates in diabetics, *J. Clin. Inv.*, 1926, ii, 608.
8. Campbell, W. R., and Markowitz, J., On the metabolism of dihydroxyacetone in pancreatic diabetes, *Am. J. Physiol.*, 1927, lxxx, 561.
9. Markowitz, J., and Campbell, W. R., The fate of dihydroxyacetone in the animal body, *Am. J. Physiol.*, 1927, lxxx, 548.
10. Campbell, W. R., and Hepburn, J., The effect of dihydroxyacetone on insulin hypoglycemia, *J. Biol. Chem.*, 1926, lxviii, 575.
11. Hewitt, J. A., and Reeves, H. G., Action of glyceric aldehyde and dihydroxyacetone on insulin hypoglycemia, *Lancet*, 1926, ii, 703.
12. Riche, J. A., and Soderstrom, G. F., Clinical calorimetry. II. The respiration calorimeter of the Russell Sage Institute of Pathology in Bellevue Hospital, *Arch. Int. Med.*, 1915, xv, 805.
13. Gephart, F. C., and Du Bois, E. F., Cinical calorimetry. III. The organization of a small metabolism ward, *Arch. Int. Med.*, 1915, xv, 829.
14. Benedict, S. R., The detection and estimation of glucose in urine, *J. Am. Med. Assn.*, 1911, lvii, 1193.
15. McCann, W. S., The effect of the ingestion of foodstuffs on the respiratory exchange in pulmonary tuberculosis, *Arch. Int. Med.*, 1921, xxviii, 847.
16. Folin, O., and Wu, H., A system of blood analysis. Supplement I. A simplified and improved method for the determination of sugar, *J. Biol. Chem.*, 1920, xli, 367.
17. Campbell, W. R., The quantitative determination of dihydroxyacetone, *J. Biol. Chem.*, 1926, lxvii, 59.
18. Van Slyke, D. D., and Cullen, G. E., Studies of acidosis. I. The bicarbonate concentration of blood plasma; its significance, and its determination as a measure of acidosis, *J. Biol. Chem.*, 1917, xxx, 289.
19. McClellan, W. S., The quantitative estimation of dihydroxyacetone in blood and urine, *J. Biol. Chem.*, 1928, lxxvi, 481.

20. Du Bois, E. F., Basal metabolism in health and disease, Philadelphia, 2nd edition, 1927, 132.
21. Lusk, G., Animal calorimetry. XXIV. Analysis of the oxidation of mixtures of carbohydrate and fat, *J. Biol. Chem.*, 1924, lix, 41.
22. Togel, O., Brezina, E., and Durig, A., Über die Kohlenhydratsparende Wirkung des Alkohols, *Biochem. Z.*, 1913, l, 296.
23. Higgins, H. L., The rapidity with which alcohol and some sugars may serve as nutriment, *Am. J. Physiol.*, 1916, xli, 258.
24. Benedict, F. G., and Carpenter, T. M., Food ingestion and energy transformation, *Carnegie Institution of Washington, Pub. No. 261*, 1918, 171.
25. Carpenter, T. M., Human metabolism with enemata of alcohol, dextrose, and levulose, *Carnegie Institution of Washington, Pub. No. 369*, 157.
26. Deuel, H. J., Jr., The respiratory metabolism following the administration of various carbohydrates, *J. Biol. Chem.*, 1927, lxxv, 367.
27. Cori, C. F., and Cori, G. T., The fate of sugar in the animal body. VIII. The influence of insulin on the utilization of glucose, fructose, and dihydroxyacetone, *J. Biol. Chem.*, 1928, lxxvi, 755.

A GENERAL REACTION OF AMINO ACIDS. II.

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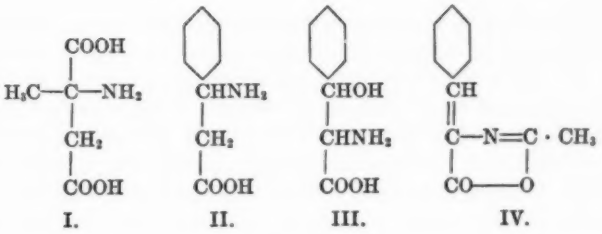
The following paper contains an account of further experiments on the reaction occurring between amino acids and a mixture of pyridine and acetic anhydride. It may be recalled that it was shown in our first paper (1) that a typical α-amino acid on being warmed with acetic anhydride and pyridine was converted into an acetylaminoacetone derivative with evolution of carbon dioxide. A β-ketonic acid was assumed to be an intermediate product.

$$R \cdot CH \cdot NH_2 \cdot COOH \rightarrow R{-}\underset{\displaystyle NH \cdot CO \cdot CH_3}{\overset{\displaystyle COOH}{\overset{|}{\underset{|}{C}}}}{-}CO \cdot CH_3 \rightarrow R \cdot \underset{\displaystyle NH \cdot CO \cdot CH_3}{\overset{\displaystyle CH}{|}} \cdot CO \cdot CH_3 + CO_2$$

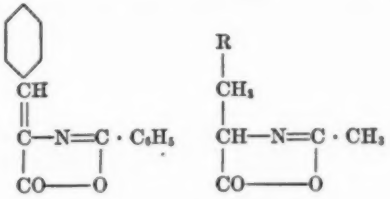
It was further shown that, if one or both of the hydrogen atoms of the amino group were substituted by alkyl groups, no typical reaction with ketone formation took place and that the same was true of an acid such as α-aminohydratropic acid which contains no unsubstituted hydrogen attached to the α-carbon atom. The bearing of these results on the mechanism of the reaction was briefly discussed. The new experiments comprise a study of the action of pyridine and acetic anhydride on a number of other types of amino acids and their derivatives. The following amino acids were found to yield substituted acetylaminoacetones which were characterized by appropriate derivatives: aspartic acid, glutamic acid, histidine, and tryptophane. On the other hand methylaspartic acid (I) gave no trace of either ketone or carbon dioxide, this result being clearly due to the absence of an unsubstituted α-hydrogen atom adjacent to the amino group. The

behavior of methylaspartic acid is analogous to that of α-amino-hydratropic acid. Phenyl-β-alanine (II), chosen as a representative of the β-amino acids, gave neither ketone nor carbon dioxide but underwent simple acetylation. Serine, typical of the β-hydroxy-α-amino acids, gave unquestionable qualitative evidence of ketone formation but the amount was small as the product appeared to be unstable. Phenylserine (III), on the other hand, gave traces of carbon dioxide but no ketone. It was largely converted into the anhydride (azlactone) of acetaminocinnamic acid (IV). The fact that α,β-unsaturated azlactones of this type are not acted upon by pyridine and acetic anhydride was confirmed by experiments with the azlactone of benzoylaminocinnamic acid (V) prepared by condensing benzaldehyde with hippuric acid. The substance was entirely unacted upon and was recovered unchanged. On the other hand Bergmann, Stern, and Witte (2) have recently described some cyclic anhydrides—so called azlactones of the saturated α-amino acids. These substances are formed by the action of acetic anhydride on amino acids and are of the type shown by the general formula (VI). Azlactones derived from leucine, phenylalanine, and aspartic acid, all react with acetic anhydride and pyridine to give carbon dioxide and the same acetylaminoketones as are furnished by the amino acids themselves. It is therefore a plausible supposition that these azlactones represent an intermediate stage in the pyridine-acetic anhydride reaction. This hypothesis offers a satisfactory explanation of the curious failure of α-alkylamino acids to undergo a reaction analogous to that of the unsubstituted amino acids for the former compounds obviously cannot yield azlactones containing a hydrogen atom in the position capable of replacement by an acetyl group. On the other hand it must be admitted that as judged by the apparent evolution of carbon dioxide, the azlactones do not react noticeably more rapidly than do the amino acids themselves so that it is not improbable that they only represent one of several intermediate steps in the reaction.

A number of peculiarities are observed in the products derived from the amino acids used in the present investigation. These will be referred to in the experimental section in order to avoid repetition.



I. II. III. IV.



V. VI.

EXPERIMENTAL.

Aspartic Acid.—When finely powdered *l*-aspartic acid (3 gm.) is warmed with acetic anhydride (20 cc.) and pyridine (10 cc.) on the steam bath, the acid slowly goes into solution and at the end of about 3 hours 85 to 95 per cent of the theoretical amount (1 mol) of carbon dioxide has been evolved. After a short distillation with steam to remove the bulk of the acetic acid and pyridine, a solution is obtained giving the typical ketone reactions including a strong iodoform test. Prolonged steam distillation results in some decomposition and diacetyl will begin to appear in the distillate. The aqueous solution on being made acid to Congo red with sulfuric acid yields scarcely anything on extraction with ether, but on repeated shaking (5 to 6 times) with butyl alcohol, the product is readily extracted. When the solvent is removed a clear amber-like gum (3.1 gm.) is obtained which shows no inclination to crystallize. The product gives a strong iodoform reaction, a deep orange-red color with sodium nitroprusside in alkaline solution, turning magenta with acetic acid. Silver, mercury, and copper salts are all reduced on warming in alkaline solution, while silver nitrate produces no precipitate in acid or neutral solution.

On addition of an excess of phenylhydrazine (3 gm.) dissolved in 10 per cent acetic acid to an aqueous solution of the gum (1 gm.) no immediate precipitation takes place but on being warmed on the water bath carbon dioxide is freely evolved and a crystalline product separates out in fair quantity (0.9 gm.). Examination of this product quickly showed that it was not a simple hydrazone of an acetylaminoketone but was the *bis*-hydrazone of diacetyl. The substance melted after recrystallization from benzene at 241–243°, had all the properties of the diacetyl derivative described by Pechmann, and gave the following results on analysis.

Analysis.

$C_{16}H_{18}N_4$.	Calculated.	C 72.1, H 6.76, N 21.0.
	Found.	" 72.0, " 6.89, " 21.2.

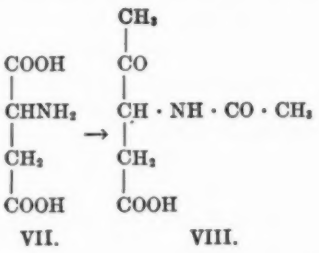
The direct formation of diacetyl itself was readily demonstrated by dissolving the original gum in dilute sulfuric acid (1:20) and distilling with steam. After a short time the distillate becomes noticeably yellow and gives all of the characteristic reactions of diacetyl.

When the gum is dissolved in dilute hydrochloric acid (10 per cent) and evaporated on the water bath a residue is obtained which contains crystals which were readily identified as ammonium chloride. The odor of diacetyl is marked during the evaporation. The non-crystalline material on being dissolved in water and extracted with ether gave a small amount of a syrupy acid agreeing in its properties with the description of β-hydroxylevulinic acid given by Wolff (3), while another portion of the syrup on being warmed on the water bath with excess of aqueous ammonia readily furnished a base, which after extraction with ether melted at 85° and gave a picrate melting at 191–192°. The base was clearly identical with tetramethylpyrazine which, as is already known, results from the action of ammonia on either diacetyl or β-hydroxylevulinic acid. The anhydrous tetramethylpyrazine gave the following results on analysis.

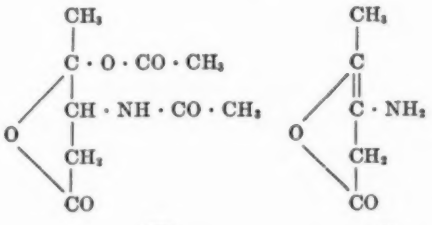
Analysis.

$C_8H_{12}N_2$.	Calculated.	C 70.6, H 8.83.
	Found.	" 70.2, " 8.97.

The foregoing results could well be interpreted on the basis of the idea that the original non-crystalline product of the action of acetic anhydride and pyridine on aspartic acid (VII) was an acetylaminoacetone derivative (VIII) such as should result from the general reaction observed by us in many analogous cases. The fact that the substance contains no amino nitrogen and that the ratio of total nitrogen to acetyl groups is exactly 1:1 is in harmony with this view. An inspection of the formula (VIII) shows that the ketone is a derivative of levulinic acid, namely



VII. VIII.



IX. X.

β-acetylaminolevulinic acid, and as is well known, levulinic acid and analogous γ-ketonic acids react with acetic anhydride to give substances of the type of acetyl-levulinic acid usually represented as γ-acetoxy-γ-valerolactone (4). There is reason to believe that a similar reaction takes place with the ketonic acid (VIII) under discussion and that it is converted by the further action of acetic anhydride into β-acetamino-γ-acetoxy-γ-valerolactone (IX). During the steam distillation and other operations connected with the working up of this product one of the acetyl groups is removed with regeneration of the ketone (VIII). Under certain conditions

it has been possible to convert β-acetylaminolevulinic acid (VIII) into a finely crystalline compound which may be designated as β-amino-α-angelica lactone (X), although it is possible though improbable, that it is a derivative of β-angelica lactone. The preparation of this substance presents difficulties since the use of acids or strong alkali is ruled out owing to the ease with which the nitrogen is split off as ammonia. It has not been found possible to establish conditions which regularly result in a successful preparation and many negative results have been encountered. When β-acetylaminolevulinic acid is dissolved in butyl alcohol containing a trace of sulfuric acid, and evaporated and subsequently dried for a week or so in a vacuum desiccator over sulfuric acid, a gummy residue is obtained. When this is warmed with a little 5 per cent sodium carbonate solution unchanged acid is dissolved and a crystalline residue of the lactone remains which is filtered off and recrystallized from boiling water. Its properties and analysis are in accord with the structure suggested. The substance is only moderately soluble in water, either hot or cold, and its aqueous solution reacts neutral to litmus. It is readily soluble in alcohol and less so in ether. It is instantly dissolved by strong hydrochloric acid but is in part recovered unchanged on dilution and evaporation. It is not decomposed by sodium carbonate but is easily decomposed by sodium hydroxide. It does not reduce Fehling's solution but reduces permanganate in alkaline solution somewhat slowly. The aqueous solution of the substance gives no iodoform reaction when treated with iodine and sodium hydroxide in the cold, but if it is first warmed with a little caustic alkali, so as to open the lactone ring, the cooled solution gives an intense iodoform reaction. On treatment with nitrous acid the compound gives nitrogen equivalent to 8.5 to 9.5 per cent in 5 minutes.

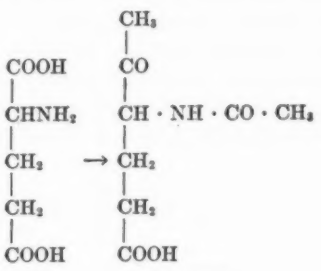
Analysis.

$C_5H_7O_2N$.	Calculated.	C 53.1, H 6.20, N 12.4.
	Found.	" 53.4, " 6.51, " 12.4.

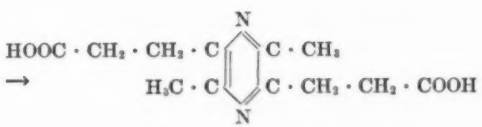
Glutamic Acid (XI).—When glutamic acid (5 gm.) is warmed with acetic anhydride (25 cc.) and pyridine (15 cc.) on the steam bath, no carbon dioxide is evolved for about half an hour and even then the evolution is slow. After 3.5 hours the amount of carbon

dioxide amounts to only 15 to 20 per cent of the amount calculated for 1 molecular proportion. This marked difference shown by glutamic acid when compared with analogous amino acids is apparently due to its conversion into pyrrolidonecarboxylic acid by the dehydrating action of the acetic anhydride. Since pyrrolidonecarboxylic acid no longer contains a free amino group such as we have found to be essential for ketone formation from amino acids, it is incapable of further reaction and accounts for the bulk of the glutamic acid originally taken. An attempt to limit the formation of pyrrolidonecarboxylic acid by adding a little acetyl chloride to the anhydride was unsuccessful.

When the reaction mixture is steam-distilled, the residue is found to contain a moderate amount of a ketone mixed with much other material. The iodoform and nitroprusside reactions are strong, and phenylhydrazine acetate gives a precipitate which readily becomes oily. Alkaline solutions of silver, copper, and mercury salts are readily reduced. It was not found possible to isolate the ketone directly, but evidence of its presence and structure (XII) were obtained through its conversion into 2, 5-dimethylpyrazine-3,6-dipropionic acid (XIII) by reactions analogous to Gabriel's pyrazine synthesis from aminoacetone.



XI. XII.



XIII.

The aqueous solution containing the acetaminoacetone derivative was made acid to Congo red and then extracted by repeated shaking with butyl alcohol. After removal of the solvent the syrupy residue was heated for an hour on the steam bath with 10 cc. of hydrochloric acid (1:1). The solution was then evaporated to remove most of the acid and then treated with excess of ammonia. After standing at room temperature for an hour the mixture was evaporated to small bulk (10 cc.). The solution which reacted acid to litmus was made more strongly acid with acetic acid and allowed to crystallize. The first crop of crystals consisted of practically pure silky needles of the pyrazine acid while subsequent crops were contaminated with glutamic acid and were discarded. The yield was only 2 to 3 per cent of the glutamic acid originally taken.

2,5-Dimethylpyrazine-3,6-dipropionic acid is a type of acid that has not hitherto been prepared. It is very sparingly soluble in cold water and only moderately soluble in boiling water. Its aqueous solution only just reddens blue litmus paper. It crystallizes from methyl alcohol, in which it is readily soluble on heating, in stout prisms while from water it separates in long needles. It is fairly soluble in ether. The acid melts at 211–213° without evolution of gas. Its saturated aqueous solution gives only a trifling brownish yellow color with ferric chloride and no immediate precipitate with either silver nitrate or mercuric chloride. When it was left standing, a finely crystalline silver salt separates in the form of heavy needles. The substance gives no diazo reaction and it contains no amino nitrogen (Van Slyke).

Analysis.

$C_{12}H_{16}O_4N_2$.	Calculated.	C 57.1, H 6.35, N 11.1.
	Found.	" 57.0, " 6.63, " 11.1.

Methylaspartic Acid.—This substance (I) was prepared from acetoacetic ester in the usual way. On being heated (1 gm.) with acetic anhydride (10 cc.) and pyridine (7 cc.) on the steam bath for several hours no carbon dioxide was evolved and when the products were worked up in the usual way no trace of any aminoacetone derivative was detected. This result is obviously in accordance with what would be expected.

Serine.—The synthetic acid (1 gm.) was heated with acetic

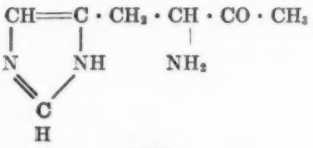
anhydride (10 cc.) and pyridine (7 cc.). After an interval of about 15 minutes carbon dioxide was freely evolved. After 4 hours the mixture was distilled with steam. The distillate contained no volatile ketone. The residue gave a strongly positive iodoform reaction but the size of the precipitate seemed small in comparison with similar tests with other amino acids. Fehling's solution was reduced on boiling while sodium nitroprusside gave a positive but rather atypical reaction. Phenylhydrazine gave small amounts of a sticky hydrazone, while boiling with *p*-nitrophenylhydrazine in 5 per cent sulfuric acid gave small amounts of an osazone giving the characteristic blue color with sodium hydroxide and alcohol. It was not found possible to prepare in crystalline form either the original product of the reaction or a satisfactory derivative. While the tests above enumerated clearly indicate that some acetaminoacetone derivative, analogous to that obtained from other amino acids, had been formed, its amount appeared small and its stability slight. Only two experiments were made with serine as the mediocre results did not seem to justify the sacrifice of larger amounts of this amino acid.

Phenylserine.—The acid (1 gm.) was warmed on the water bath with acetic anhydride (7 cc.) and pyridine (5 cc.) for 8 hours. A slight evolution of carbon dioxide took place but on steam distillation neither distillate nor residue gave any reactions indicative of ketone formation. Crystals separated from the nonvolatile aqueous residue which melted at 152°. They were identified by properties and by analysis as the "azlactone" anhydride of acetaminocinnamic acid (IV) first described by Erlenmeyer and Früstück (5). On being dissolved in warm sodium hydroxide and precipitated with hydrochloric acid, acetaminocinnamic acid melting at 190° was obtained.

Phenyl-β-Alanine.—This amino acid (1 gm.) was treated exactly as described above in the case of phenylserine. No carbon dioxide was evolved and no ketone formation took place. The aqueous residue gave a large yield of acetyl-phenyl-β-alanine, which after crystallizing from acetic acid melted at 161–162° as described by Posner (6).

Histidine.—Experiments were made with either the free histidine base or the dihydrochloride without disclosing any difference. On being warmed (1 gm.) on the water bath with

acetic anhydride (7 cc.) and pyridine (5 cc.), carbon dioxide was evolved freely after about 10 minutes and in the course of 2 hours 93.5 to 97 per cent of the amount of carbon dioxide calculated for 1 molecular proportion was obtained. After steam distillation the residue was found to give intense iodoform and nitroprusside reactions and to reduce alkaline solutions of copper, silver, and mercury salts. Phenylhydrazine acetate gave an unattractive hydrazone slowly separating in minute needles. Semicarbazide and aminoguanidine gave no crystalline derivatives. Since the acetylaminoketone appeared to crystallize with difficulty it was decided to remove the acetyl group by evaporation with 10 per cent hydrochloric acid. The concentrated solution on standing deposited stout colorless prisms melting at 205–206° which gave results on analysis in satisfactory agreement with the expected dihydrochloride of (4)-imidazolyl-(3)-amino-butanone (2) (XIV).



XIV.

Analysis.

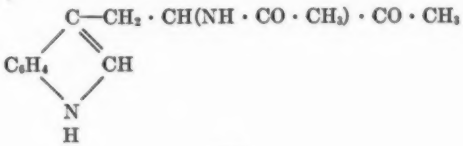
$C_7H_{11}ON_3 \cdot 2HCl$.

Calculated.	C 37.2, H 5.75, N 18.6,	amino N 6.19, Cl 31.4.
Found.	" 37.0, " 6.01, " 18.7,	" " 5.95, " 31.7.

The yield of crystalline hydrochloride amounts to about 60 per cent of the theoretical amount but much additional product remains in the mother liquor. It gives the reactions described above typical of aminoacetone derivatives and also an intense diazo reaction while on treatment with alkali yields a pyrazine derivative which will be further examined. It will be noticed that the base only differs from β-iminazolethylamine by the substitution of a hydrogen atom by an acetyl group but unlike the latter substance it was found by Dr. C. Lieb scarcely to affect the blood pressure when doses of from 1 to 20 mg. were injected intravenously into a cat.

Tryptophane.—This amino acid (1 gm.) was treated as usual with acetic anhydride (6 cc.) and pyridine (4 cc.). The reaction

was fairly vigorous and carbon dioxide was evolved freely. On steam distillation a little tar separated and was removed by filtration. On concentration of the aqueous solution, a clear yellow oil, moderately soluble in water, was obtained. It did not crystallize. Bromine water gives a thick yellow precipitate while iodine and sodium hydroxide give a deep wine-red color with only a small separation of iodoform. Fehling's solution is reduced on boiling with development of an indole-like odor. The glyoxylic reaction is quite unlike tryptophane; at first a red color develops which turns to olive-green. On addition of an excess of phenylhydrazine in 5 per cent acetic acid to the solution, a thick yellow hydrazone separates out which is sparingly soluble in water but very soluble in organic solvents, with the exception of petroleum ether. It may be crystallized from a little benzene by addition of petroleum ether. The hydrazone on analysis gave results corresponding fairly closely with those calculated for the hydrazone of an acetyl derivative of the anticipated ketone (XV).



XV.

Analysis.

$C_{22}H_{24}O_2N_4$.	Calculated.	C 70.2, H 6.39, N 14.9.
	Found.	" 69.9, " 6.65, " 14.5.

The experiments with tryptophane must only be regarded as of a preliminary character.

Azlactones.—The azlactones of leucine, phenylalanine, and aspartic acid were prepared by the method given by Bergmann, Stern, and Witte (2). They behaved with pyridine and acetic anhydride precisely like the amino acids from which they were prepared and furnished the same acetylaminoacetone derivatives, the characterization of which has already been described. The details of these experiments therefore need no minute description. On the other hand the azlactone of benzoylaminocinnamic acid gave neither carbon dioxide nor ketone as was to be anticipated.

BIBLIOGRAPHY.

1. Dakin, H. D., and West, R., *J. Biol. Chem.*, 1928, lxxviii, 91.
2. Bergmann, M., Stern, F., and Witte, C., *Ann. Chem.*, 1926, cdxlix, 277.
3. Wolff, L., *Ann. Chem.*, 1891, cclxiv, 235.
4. Bredt, A., *Ann. Chem.*, 1890, cclvi, 314.
5. Erlenmeyer, E., and Früstück, E., *Ann. Chem.*, 1895, cclxxxiv, 36.
6. Posner, T., *Ber. chem. Ges.*, 1905, xxxviii, 2316.

SOME AROMATIC DERIVATIVES OF SUBSTITUTED ACETYLAMINOACETONES.

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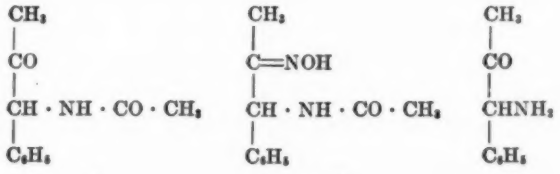
In preceding papers (1) we have described a reaction by which α-amino acids may be converted into acetylaminoacetone derivatives by the action of acetic anhydride and pyridine. The researches of Gabriel and his coworkers and others furnish abundant evidence of the great reactivity of aminoacetone and its homologues and their value in synthetic work. Since a number of these ketone derivatives are now easily prepared from readily accessible amino acids it appeared worth while to examine some of their transformations in order to illustrate their possibilities for preparative purposes. The present communication simply deals with some of the substances prepared from aromatic α-amino acids such as phenyl-α-aminoacetic acid, phenylalanine, and tyrosine. The amino acids were first converted into their corresponding acetylaminoacetone derivatives by the action of acetic anhydride and pyridine as already described in our first paper. The further transformations of the acetylaminoacetone compounds follow well established reactions and offer nothing essentially new as regards methods.



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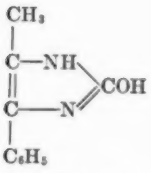

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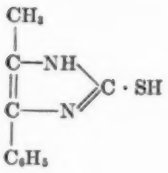

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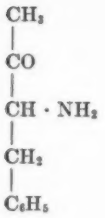

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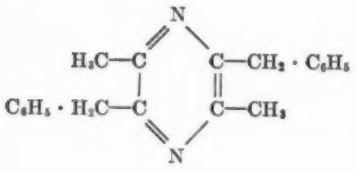

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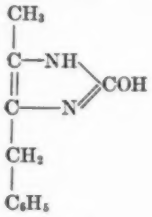

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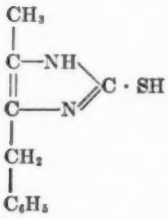

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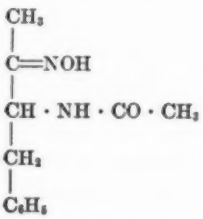

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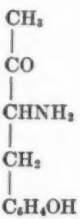

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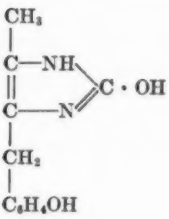

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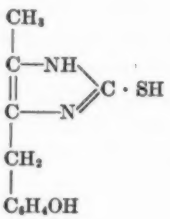

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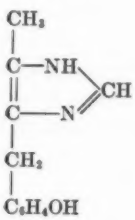

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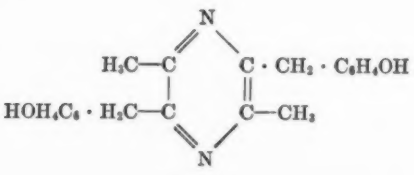

XV.

EXPERIMENTAL.

α-Phenyl-α-Acetaminoacetone (*I*).—Details of the preparation of this substance from phenylaminoacetic acid have already been given (1) together with its conversion by hydrolysis into the hydrochloride of α-phenyl-α-aminoacetone (III) already described by Kolb (2).

α-Phenyl-α-Acetaminoacetoxime (*II*).—The ketone (I) (1 gm.) was dissolved in warm alcohol (3 cc.) and treated with hydroxylamine hydrochloride (1 gm.) dissolved in a little water together with sodium bicarbonate (0.8 gm.). The oxime quickly crystallizes out in the form of nacreous crystals, very sparingly soluble in water and moderately soluble in alcohol. It melts at 152°. Yield 0.8 gm.

Analysis.

$C_{11}H_{14}O_2N_2$.	Calculated.	C 64.1, H 6.80.
	Found.	" 64.3, " 6.93.

The oxime just described on being dissolved in alcohol and reduced with sodium and acetic acid gives a base with an extremely powerful depressor action which will be further investigated.

4-Phenyl-5-Methyliminazolone.—α-Phenyl-α-aminoacetone hydrochloride (III) (1 gm.) was dissolved in 5 cc. of water and warmed on the water bath with potassium cyanate (1 gm.). The iminazolone soon begins to separate out in the form of sandy rosettes of needles melting at 287–289°. Yield 0.8 gm. The substance is almost insoluble in water and in sodium carbonate solution. On treatment with diazo salts in sodium carbonate solution only a faint yellow color is produced.

Analysis.

$C_{10}H_{10}ON_2$.	Calculated.	C 68.9, H 5.75, N 16.1.
	Found.	" 68.9, " 5.90, " 16.0.

4-Phenyl-5-Methyl-2-Thioliminazole(*V*).—α-Phenyl-α-aminoacetone hydrochloride (1 gm.) was dissolved in 5 cc. of water and heated in a covered dish on the steam bath with potassium thiocyanate for a couple of hours. After a short time slightly yellow shining needles begin to separate. The yield was 0.64 gm. The substance is very sparingly soluble in hot water or alcohol but can

XUM

be recrystallized from the latter when it separates in the form of thin shining plates. It softens slightly at above 250° and melts rather indefinitely at 290–295°. It gives a moderately strong orange-yellow with alkaline diazo salts.

Analysis.

$C_{10}H_{10}N_2S$.	Calculated.	N 14.7, S 16.8.
	Found.	" 14.8, " 16.2.

This substance and the preceding one are presumably identical with the compounds obtained by Behr-Bregowski (3) from the isomeric amidoethyl-phenyl ketone hydrochloride.

α-Benzyl-α-Aminoacetone (*VI*).—The hydrochloride of this base was easily obtained by heating its acetyl derivative (3 gm.), prepared from phenylalanine as previously described, with 10 per cent hydrochloric acid. After being heated for an hour on the steam bath, the solution was evaporated to small bulk and then taken up in cold water. A trace of oily matter was separated by filtration and the filtrate concentrated to a thin syrup. After it has stood crystals readily separate in good yield. The substance forms hexagonal platelets extremely soluble in both water and alcohol and melts at 130°.

Analysis.

$C_{10}H_{13}ON \cdot HCl$.	Calculated.	Total N 7.03, amino N 7.03.
	Found.	" " 7.10, " " 7.09.

2,5-Dibenzyl-3,6-Dimethylpyrazine (*VII*).—The hydrochloride of α-benzyl-α-aminoacetone (VI) (1 gm.) was dissolved in 5 cc. of water and concentrated aqueous ammonia added (5 cc.) and the mixture allowed to stand in an open dish overnight. Oily droplets separated out which later changed to long silky needles. The crystals were dissolved in excess of hydrochloric acid, filtered from a little oily impurity, and then extracted with ether. The acid extract was disregarded and the solution again extracted with ether after being made alkaline with sodium hydroxide. The ether on evaporation left a mass of crystals which melted in the crude condition at about 85°. After being dried on a porous plate and recrystallized from a few drops of alcohol a small yield of good crystals, both plates and needles, melting at 92–94°, was obtained. The base is sparingly soluble in water and extremely soluble in

alcohol and ether and less so in petroleum ether. The base is volatile in steam and has a slight hyacinth-like odor. The vapor turns red litmus blue but it is not a strong base and requires a considerable excess of hydrochloric acid to dissolve it. The hydrochloride is very soluble and like the analogous 2,5-diphenyl-3,6-dimethylpyrazine hydrochloride, its solution in hydrochloric acid is decidedly yellow. The base gives a picrate crystallizing in needles and a thick precipitate with mercuric chloride which crystallized in needles from dilute hydrochloric acid.

Analysis.

$C_{10}H_{20}N_2$.	Calculated.	C 83.3, H 6.95, N 9.73.
	Found.	" 82.9, " 7.20, " 10.1.

It may be mentioned that on distillation of α-benzyl-α-aminoacetone hydrochloride with mercuric chloride and concentrated sodium hydroxide by the method so commonly successful with non-aromatic pyrazines only a trace of solid pyrazine was found in the distillate, most of the volatile base being ammonia.

4-Benzyl-5-Methyliminazolone (VIII).—The preparation of this substance from α-benzyl-α-aminoacetone hydrochloride and potassium cyanate was carried out exactly as for its lower homologue (IV). The yield was about 65 per cent. The compound is sparingly soluble in water and on heating begins to darken above 250° and turns to a black mass indefinitely around 270°. The diazo reaction is of negligible intensity.

Analysis.

$C_{11}H_{12}ON_2$.	Calculated.	C 70.2, H 6.38.
	Found.	" 70.0, " 6.51.

4-Benzyl-5-Methyl-2-Thioliminazole (IX).—1 gm. of α-benzyl-α-aminoacetone hydrochloride gave, on being warmed with an equal weight of potassium thiocyanate in 10 cc. of water, 0.72 gm. of the above compound which crystallized in silky opaque needles. It is very insoluble in water and melts at 279–280°, darkening a degree or two before melting. It gives a very trivial reaction with diazo salts in alkaline solution but if it is first boiled with ferric chloride to oxidize the thiol group, the solution on being made alkaline with soda gives an intense diazo reaction. This result is in accordance with Pyman's views of the Pauly reaction ac-

XUM

cording to which a replaceable hydrogen in position (2), (4), or (5) is essential for a typical positive reaction.

Analysis.

$C_{11}H_{12}N_2S$.	Calculated.	N 13.7.
	Found.	" 13.6.

α-p-Hydroxybenzyl-α-Acetaminoacetoxime (*X*).—This substance is readily obtained from *p*-hydroxybenzyl-acetaminoacetone, the preparation of which from tyrosine has already been described. When the ketone (1 gm.) is dissolved in warm water (5 cc.) and an excess of hydroxylamine hydrochloride added, separation of the oxime begins at once with liberation of free hydrochloric acid. Sodium carbonate solution was then added by degrees until the reaction to Congo red was no longer acid. The yield of oxime is almost the theoretical amount. It is sparingly soluble in water and readily soluble in alcohol. It may be crystallized conveniently from methyl alcohol when it separates in tufts of fine silky needles, resembling tyrosine, and melts at 189–190°. It is readily reduced by sodium amalgam in acid solution.

Analysis.

$C_{12}H_{16}O_2N_2$.	Calculated.	C 65.5, H 7.27.
	Found.	" 65.6, " 7.41.

α-p-Hydroxybenzyl-α-Aminoacetone Hydrochloride (*XI*).—When the acetyl derivative of this compound, prepared as already described from tyrosine (1) was hydrolyzed with 10 per cent hydrochloric acid on the steam bath, the hydrochloride of the base was obtained on evaporation. It is extremely soluble in water and in alcohol but is precipitated from its alcoholic solution by ether as a slightly yellow deliquescent syrup which did not crystallize readily. It was not analyzed. The reaction typical of aminoacetone derivatives were all positive.

4-p-Hydroxybenzyl-5-Methyliminazolone (*XII*).—The preceding hydrochloride (1 gm.) on being warmed with potassium cyanate (1 gm.) in aqueous solution (10 cc.) gave 0.4 gm. of the iminazolone derivative after being made acid to Congo red with sulfuric acid. The substance crystallizes in large stout prisms, is very sparingly soluble in cold or hot water, somewhat more soluble in alcohol, and

freely soluble in glacial acetic acid. It was recrystallized from aqueous methyl alcohol and melted at 243–244°.

Analysis.

$C_{11}H_{12}O_2N_2$.	Calculated.	C 64.7, H 5.88.
	Found.	" 64.2, " 6.01.

4-p-Hydroxybenzyl-5-Methyl-2-Thioliminazole (*XIII*).—The action of aqueous potassium thiocyanate on the aminoacetone derivative (XI) is rather slow and 2 or 3 hours heating on the steam bath is requisite. The conditions were those previously described for homologous substances. The thioliminazole separates out in crystals carrying a very faint yellowish tinge. It is very sparingly soluble in water, alcohol, or acetic acid. On recrystallization from methyl alcohol cubes and columnar prisms were obtained which darkened slightly above 260° and melted rather indefinitely at 273–275°.

Analysis.

$C_{11}H_{12}ON_2S$.	Calculated.	N 12.8, S 14.5.
	Found.	" 12.7, " 14.3.

The substance is most easily purified by being dissolved in dilute sodium hydroxide and precipitated with acetic acid. The alcoholic solution gives a blood-red color with gold chloride, turning to a dirty gray precipitate. Aqueous iodine is at first decolorized, then with excess gives a chocolate-brown precipitate turning almost black. When the substance (1.75 gm.) is boiled with ferric chloride (12 gm.) in 50 per cent alcohol solution, the sulfur is removed. The sulfur-free iminazole is obtained by removal of the iron with sodium hydroxide and acidification of the filtrate with acetic acid. It is a fine creamy white solid, very sparingly soluble in water, sodium carbonate solution, alcohol, or acetic acid. It does not melt below 290° but only darkens slightly. The yield was 0.4 gm. The substance was only analyzed for nitrogen (14.7 per cent) but undoubtedly has the structure corresponding to 4-*p*-hydroxybenzyl-5-methyliminazole (XIV).

2, 5-p-Dihydroxybenzyl-3, 6-Dimethylpyrazine (*XV*).—When the hydrochloride of (XI) is dissolved in water, a slight excess of aqueous ammonia added, and the mixture allowed to stand overnight in an open dish, a sparingly soluble precipitate separates in

about 40 per cent yield. It is easily purified by being dissolved in dilute sodium hydroxide and when carbon dioxide is passed into the solution it is precipitated as a very light powder with a faint tinge of buff color. It dissolves in concentrated sulfuric acid to give an orange-yellow solution and is soluble in warm hydrochloric acid if not too dilute. It gives no amino nitrogen on treatment with nitrous acid and apparently is the normally constituted pyrazine.

Analysis.

$C_{20}H_{20}O_2N_2$.	Calculated.	C 75.0, H 6.25, N 8.75.
	Found.	" 74.9, " 6.47, " 8.60.

BIBLIOGRAPHY.

1. Dakin, H. D., and West, R., *J. Biol. Chem.*, 1928, lxxviii, 91, 745.
2. Kolb, A., *Ann. Chem.*, 1896, ccxci, 276.
3. Behr-Bregowski, L., *Ber. chem. Ges.*, 1897, xxx, 1515.

STUDIES OF GAS AND ELECTROLYTE EQUILIBRIA IN BLOOD.

X. THE SOLUBILITY OF CARBON DIOXIDE AT 38° IN WATER, SALT SOLUTION, SERUM, AND BLOOD CELLS.

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Interest in the magnitude of the solubility of carbonic acid in biological fluids dates from the beginning of inquiries into the manner of transport of CO_2. Of late years it has been of especial importance in the calculations of the pH of bicarbonate-containing fluids by Hasselbalch's equation, $pH = pK_1' + \log \frac{[BHCO_3].}{[H_2CO_3]}$ The physically dissolved CO_2, expressed as H_2CO_3, is calculated from the CO_2 tension in the atmosphere with which the blood or solution is equilibrated by means of the solubility coefficient of the gas in the solution.[1]

[1] The "physically dissolved CO_2" taken up by water solutions is in part hydrated to H_2CO_3; consequently it is in part altered by chemical reaction with the solvent H_2O. According to Buytendyk *et al.* (1927) the greater part of the physically dissolved CO_2 at room temperature does remain as the anhydride, only a small portion changing to H_2CO_3. The convention by which the total hydrated plus anhydrous CO_2 is at the same time called "physically dissolved" and formulated as H_2CO_3 is inconsistent and inexact. The convention is, however, convenient, and causes no errors if the H_2CO_3 symbol is used consistently with the same meaning: we shall accordingly continue to use it.

Bunsen's solubility coefficient, customarily symbolized as α, expresses the cc. of a gas, measured at 0°, 760 mm., dissolved per cc. of solution when the latter is under 1 conventional atmosphere (760 mm.) of pressure. The tension of a gas being known, the volume of gas dissolved per unit volume of solution is calculated as $\alpha \times$ atmospheres of tension. In calculating pH by Hasselbalch's equation an error of 2 per cent in α_{CO_2} (*e.g.* caused by using $\alpha = 0.52$ instead of 0.51) causes an error of approximately 0.01 in the calculation of a blood serum pH value.

Determinations of the solubility of CO_2 in water and rather concentrated salt solutions at various temperatures have been made by Bohr (1891, 1899, 1905), Bohr and Bock (1891), Setschenow (1892), Geffcken (1904), and Just (1901).

In serum and blood, however, the difficulty of distinguishing between CO_2 combined with alkali (as bicarbonate) and CO_2 "physically dissolved" has deterred investigators from direct determinations of the latter. The CO_2 solubility coefficients for blood and blood serum in general use for the past 20 years have been those estimated indirectly from oxygen and hydrogen solubilities by Bohr (1905). He followed the general assumption, which he accredited to Zuntz, that different gases in a given aqueous solution, such as blood serum, will each show the same percentage of its solubility in pure water. Bohr found that oxygen was 97.5 per cent as soluble in serum as in water, and that hydrogen was 92 per cent as soluble in whole blood as in water. He assumed that CO_2, like O_2, was 97.5 per cent as soluble in serum as in water, and like H_2 was 92 per cent as soluble in whole blood as in water. On these assumptions he calculated the solubility coefficients of CO_2 at 38° to be $0.975 \times 0.555 = 0.541$ in plasma, and $0.92 \times 0.555 = 0.511$ in whole blood, 0.555 being taken as the solubility coefficient in water. Assuming one-third of the blood volume to be cells, he estimated the CO_2 solubility coefficient in them to be 0.45, or 81 per cent of the solubility in water. Fahr (1912) in a paper published from Bohr's laboratory after Bohr's untimely death, found by direct determinations on centrifuged cells that the relative solubility of H_2 in them was not 81, but 86 to 90 per cent of its solubility in water. Nevertheless Bohr's original estimation of α_{CO_2} in cells as 81 per cent of that in water has continued to find acceptance by nearly all investigators who have had occasion to use the value.

Peculiarly, the combination of errors affecting the different factors by which Bohr reached his value for α_{CO_2} in cells yielded, within the limits of probable variation in cells from different bloods, the same value for α_{CO_2} at which we have arrived in the present paper. For α_{CO_2} in serum, however, we obtain a value much lower than his.

The assumption that different gases show the same relative solubilities in different solutions has not proved exact ("relative solubility" $= \left(\frac{\text{(observed solubility)}}{\text{(solubility in water)}}\right)$. The data in Table I indicate the extent to which the relative solubilities of different gases can vary in solutions in general, and in blood in particular. If the relative solubilities of the different gases in the same solvent were equal, the values in each horizontal line of Table I would all be alike.

We have determined the solubility of CO_2 directly in acidified serum, without dilution except that caused by addition of 1 cc. of concentrated lactic acid per 100 cc. of serum. In the case of cells

TABLE I.

Relative Solubilities of Various Gases in Different Solvents.

The data, except for blood, plasma, and cells, are from the Landolt-Börnstein "Tabellen," and are for 20° temperature. The blood data are for 37–38° unless otherwise indicated.

Gas	O_2	N_2	CO	H_2	N_2O	CO_2	Acetylene.
Solvent.	$\frac{\alpha \text{ solvent}}{\alpha H_2O}$	$\frac{\alpha \text{ solvent}}{\alpha H_2O}$	$\frac{\alpha \text{ solvent}}{\alpha H_2O}$	$\frac{\alpha \text{ solvent}}{\alpha H_2O}$	$\frac{\alpha \text{ solvent}}{\alpha H_2O}$	$\frac{\alpha \text{ solvent}}{\alpha H_2O}$	$\frac{\alpha \text{ solvent}}{\alpha H_2O}$
1 M NaCl	0.724	0.601		0.785		0.794	
1 " KCl	0.737			0.798	0.800	0.848	
1 " HCl	0.932			0.928	0.956	0.968	
0.5 " H_2SO_4	0.894			0.918	0.914	0.932	
1 " KOH	0.670			0.736	0.734		
1 " NaOH	0.662			0.721			
1 " sucrose	0.554			0.680		0.740	
Benzene		7.10	7.10	3.80		2.94	
Methyl alcohol	7.67	8.66	8.13	4.75		4.65	
Ethyl "	7.70	8.77	8.00	3.75		3.28	
Amyl "		7.50	7.13	1.51		2.22	
" acetate		9.44	8.90	3.89		4.99	
Acetone	6.91	8.94	9.26	3.85		7.62	
Ethyl ether				6.33		5.11	
Whole blood		1.27* to 1.52		0.85† to 0.91	112‡		
Plasma or serum	0.975§	0.97¶	0.80 (15°)‖ 0.72 (37°)	0.94† to 0.95	0.975‡	0.934**	0.98††
Cells				0.86† to 0.90		0.79**	0.99††

* Bohr (1897).
† Fahr (1912).
‡ Siebeck (1909).
§ Bohr (1905).
¶ Stoddard (1926–27).
‖ O'Brien and Parker (1922).
** Present paper.
†† Schoen (1923).

it was impossible to work with undiluted material because of its viscosity. We have consequently determined the solubility of CO_2 in acidified cells diluted with 3 or more volumes of water, and estimated the solubility in undiluted cells by extrapolation.

We have also studied the effects of salts and acids in water solution on the solubility of CO_2 in order to estimate the effect of the electrolytes on the solubility of the gas in blood.

EXPERIMENTAL TECHNIQUE.

Preparation of Material.

For the determination of the solubility of CO_2 in *water* ordinary distilled water was acidified with HCl to 0.01 N concentration in order to overcome the trace of alkali usually present. The concentration of HCl used is sufficient to affect the solubility coefficient by only 1 part in 2000.

The solutions of acids were standardized by titration with phenolphthalein as indicator. The lactic acid solutions were boiled in order to break up the anhydride.

Of the *salt solutions* reported in Table IV, the NaCl and KCl solutions were prepared by dissolving weighed portions of the salts in 0.01 N hydrochloric acid. The lactate solutions were made by adding known excesses of lactic acid to solutions of $KHCO_3$ and $NaHCO_3$. The KH_2PO_4 solution was prepared by dissolving weighed amounts of the salt in standard dilute H_3PO_4. The NaH_2PO_4 solutions were prepared by addition of NaOH to standard H_3PO_4. The acid oxalate solutions were prepared from $K_2C_2O_4$ plus equimolar weights of free oxalic acid crystals. In all the salt solutions the pH was determined and found to be below 3.5.

Serum and cells were obtained by centrifugation of defibrinated blood. The *plasma* reported in Table VII was obtained from whole blood which had been treated with approximately 0.15 per cent of potassium oxalate, equivalent to about 0.2 gm. per 100 cc. of plasma present. The two plasmas reported in Table VII received about 0.5 per cent of potassium oxalate.

Saturation with CO_2.

Two procedures were used for saturating solutions with CO_2 gas. One consisted of bubbling it through the solution, as shown

in Fig. 1. In the other procedure, which we shall call the "tonometer saturation," a relatively small volume of solution was rotated in a large vessel filled with the gas at atmospheric pressure. Both procedures gave identical results with solutions of acids and salts. Only the tonometer saturation was used with plasma and

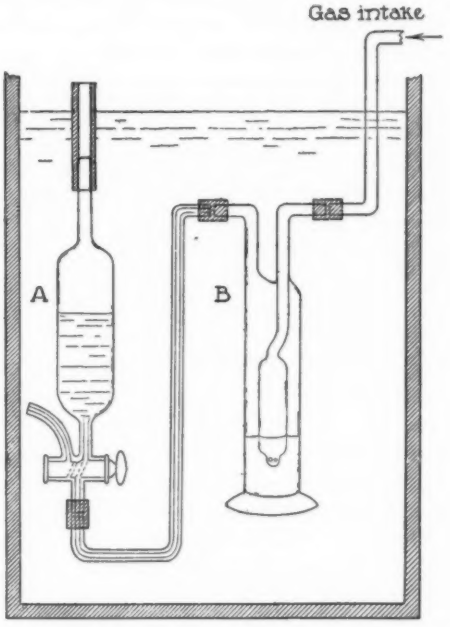

FIG. 1. Apparatus used for saturation with gas by bubbling method.

with cell solutions, saturation by bubbling being difficult because of frothing.

Saturation by Bubbling.—The solution, usually 25 to 50 cc., was placed in the saturating tube shown in Fig. 1, the lower end of which was connected through a wash bottle with a Kipp CO_2 generator. The outlet from the upper end was sufficiently wide (6 mm. bore) to prevent back pressure.

The tube and wash bottle were immersed in a constant tempera-

ture water bath[2] maintained at 38.0°±0.1°. After the solution had reached the temperature of the bath, CO_2 was bubbled through it rapidly for 30 minutes, which was twice the time found sufficient to complete the saturation. At the end of this time the cock at the bottom of the tube was closed and the wash bottle, *B*, was replaced by a mercury reservoir. Without removing the saturating tube from the bath, the free gas was driven completely out of it by admitting mercury underneath the solution, until the latter was driven up into the rubber outlet tube. The rubber tube was then closed with a clamp, and the solution was ready for determination of its CO_2 content.

The CO_2 from the Kipp generator was tested by absorbing 1 liter with alkali. The unabsorbed residue was always less than 1 part in 2000 of the CO_2.

Saturation in Rotating Tonometer.—The technique used was that described by Austin *et al.* (1922) as their "First Saturation Method," with a double-chambered tonometer to permit separation of the liquid and gas phases after saturation was finished. The gas phase was renewed once during the saturation

Analyses.

The *CO_2 contents* of the solutions were determined in the manometric gas apparatus of Van Slyke and Neill (1924). The special 5 cc. pipette and technique for transfer of the solution from the saturating tube to the manometric gas apparatus, described in section XII of Van Slyke and Neill's paper were employed. The analyses were carried out in a Van Slyke-Neill gas apparatus with an extraction chamber of 100 cc. capacity instead of the usual 50 cc. The CO_2 gas extracted from each 5 cc. sample of saturated solution was brought to 4 cc. volume for measurement of its pressure, which was determined by the difference between the manometer readings before and after absorption of the CO_2 with NaOH solution. The pressures measured were in the neighborhood of 500 mm. when the solutions had been saturated with pure CO_2 at 38°. Duplicate analyses at the same tem-

[2] The thermometers in the bath were calibrated by comparison with two Bureau of Standards thermometers. The temperature was found not to vary in any part of the bath by more than 0.1° from 38°.

perature usually agreed within 1 mm. of pressure. The CO_2 contents of the solutions were calculated from the pressures by means of the factors recently published by Van Slyke and Sendroy (1927).

Of the solutions equilibrated with CO_2 under tensions of only 40 to 50 mm. (Table XII), 15 cc. samples were taken for analysis. The extracted CO_2 was brought for measurement to a volume of 1.000 cc. and gave pressures in the neighborhood of 350 mm.

All CO_2 determinations were performed in duplicate or triplicate. Except in Table XII, we give only the mean result of the analyses of each solution. With this exception, whenever two analyses are reported for the same solution, they indicate that separate portions were saturated independently with CO_2.

The *water contents* of sera, plasmas, and cell solutions were determined by drying samples of 2.000 cc. volume overnight at 110° and determining the loss of weight. The water contents of the salt and acid solutions were estimated from the specific gravity; from the total weight of a cc. of solution the known weight of salt or acid in it was subtracted to obtain the weight of water.

Chlorides were determined by the wet ashing method of Van Slyke (1923–24).

The *protein* contents of the plasmas and sera were estimated by multiplying with 6.25 the total nitrogen determined by the Kjeldahl method. The protein content of cells was estimated as $N \times 5.78$.

The *fat* was determined gravimetrically on 10 cc. samples by the Röse-Gottlieb method.[3]

The *total base* in serum and plasma was determined by the gasometric method of Van Slyke, Hiller, and Berthelsen (1927).

Sodium and potassium in cells were determined by ashing 5 cc. samples with nitric acid, converting the ash into sulfates, removing CO_2, Ca, and PO_4 with ammonium carbonate and barium hydroxide. The Na and K sulfates were weighed, and the K determined as chloroplatinate, as outlined in the "Methods of Analysis of the Association of Official Agricultural Chemists."

[3] The Röse-Gottlieb method was used as described on p. 262 of "Official and tentative methods of analysis of the Association of Official Agricultural Chemists," Washington, 1924.

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Calculations.

The CO_2 tension in solutions saturated with pure CO_2 at atmospheric pressure was calculated as $B - W$, where B is the barometric pressure and W the vapor tension of water at the temperature of saturation (at 38° W is 49.7 mm.). In solutions saturated with a mixture of CO_2 and H_2 the CO_2 content of the dry gas mixture was determined by Haldane analysis as modified by Y. Henderson (1918), and the CO_2 tension, p, was calculated by the usual formula

TABLE II.

Solubility of CO_2 at 38° in Water Acidified by Addition of HCl to 0.01 N Concentration.

H_2O content of solution = 0.997 gm. per liter at 24°.

Method of saturation.	CO_2 content.*	CO_2 tension.	α	α^0
	vol. per cent	*mm.*	*cc. CO_2 per cc. solution*	*cc. CO_2 per gm. H_2O*
T	51.53	715.1	0.5452	0.5468
B	50.48	700.8	0.5450	0.5466
T	51.23	712.0	0.5444	0.5460
B	52.02	721.8	0.5453	0.5469
T	52.10	723.0	0.5453	0.5469
B	51.90	719.3	0.5459	0.5475
Average			0.5452	0.5468
Average estimated for pure H_2O by correcting for depression of 0.0003 in α due to 0.01 mol of HCl present			0.5455	0.5471

* The CO_2 contents given are as determined in solutions cooled to room temperature of 22–24°. The volume shrinkage is corrected for in calculating α by Equation 2.

$$(1) \qquad p = \frac{\text{per cent } CO_2 \text{ in gas}}{100} \times (B - W)$$

Bunsen's solubility coefficient, α, was calculated by the formula

$$(2) \qquad \alpha = \frac{760}{p} \times \frac{[CO_2]}{100} \times \frac{V_R}{V_{38°}}$$

TABLE III.

Solubilities of CO_2 at 38° in Solutions of Hydrochloric, Lactic, and Phosphoric Acids.

Method of saturation.	Acid.	Concentration at 22°.	H_2O content at 22°.*	CO_2 content.	CO_2 tension.	α	α^0	$\Delta\alpha$		$\Delta\alpha^0$	
								Observed.	Per mol acid present per liter.	Observed.	Per mol acid present per kg. H_2O.
		mM per l.	*gm. per cc.*	*vol. per cent*	*mm.*	*cc. CO_2 per cc. solution*	*cc. CO_2 per gm. H_2O*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per gm. H_2O*	*cc. CO_2 per gm. H_2O*
B	HCl	256	0.993	51.36	721.8	0.5384	0.5427	0.0071	0.028	0.0044	0.0017
"	"	300	0.992	51.31	723.7	0.5364	0.5407	0.0091	0.030	0.0064	0.0021
Average									0.029		0.0019
B	Lactic.	100	0.994	52.05	716	0.550	0.553				
T	"	100	0.994	52.18	717	0.549	0.552				
"	"	100	0.994	51.55	716	0.545	0.548				
"	"	100	0.994	51.83	718	0.546	0.549				
"	"	100	0.994	51.21	709	0.5465	0.550				
Average for 100 mM						0.547	0.550	−0 0015	−0.015	−0.0029	−0.0029
B	Lactic.	150	0.987	51.22	712.9	0.5436	0.5508	±0.000	±0.000	−0.0053	−0.0035
"	"	150	0.987	51.68	714.4	0.5473	0.5540				
"	"	300	0.976	51.20	712.9	0.5434	0.5569	+0.0021	+0.007	−0.0098	−0.0033
Average									−0.003		−0.0032
B	H_3PO_4	150	0.990	51.61	723.7	0.5396	0.5451	0.0059	0.0039	0.0020	0.013
"	"	300	0.983	50.86	723.7	0.5317	0.5410	0.0138	0.0046	0.0061	0.020
Average									0.0044		0.017

* H_2O contents were calculated from specific gravity minus the content of acid in gm. per cc. $\Delta\alpha$ and $\Delta\alpha^0$ indicate depressions of solubility below those in water.

TABLE IV — *Solubility of* CO_2 *in Salt*

Method of saturation.	Salt. Kind.	Salt. Concentration.		Free acid. Kind.	Free acid. Concentration.		H_2O content at 22 — 24°.	CO_2 content at 22 — 24°.	CO_2 ab... atm... 38°.
		mols. per l.	*mols. per kg. H_2O*		*mols. per l.*	*mols. per kg. H_2O*	*gm. per cc.*	*vol. per cent*	*mm.*
B	NaCl	0.150	0.1508	HCl	0.010	0.010	0.995	50.56	721.
T	"	0.150	0.1508	"	0.010	0.010	0.995	49.85	715.
B	"	0.300	0.3024	"	0.010	0.010	0.992	48.80	721.
T	"	0.300	0.3024	"	0.010	0.010	0.992	48.33	715.
B	KCl	0.150	0.1511	HCl	0.010	0.010	0.993	49.32	700.
T	"	0.150	0.1511	"	0.010	0.010	0.993	49.80	709.
B	"	0.300	0.3031	"	0.010	0.010	0.990	48.22	700.
T	"	0.300	0.3031	"	0.010	0.010	0.990	48.61	709.
B	Na lactate.	0.150	0.1526	Lactic.	0.100	0.102	0.983	48.30	700.
T	" "	0.150	0.1537	"	0.150	0.154	0.976	48.78	707.
B	" "	0.300	0.3090	"	0.100	0.103	0.971	46.53	700.
T	" "	0.300	0.3137	"	0.300	0.314	0.956	46.53	707.
B	K lactate.	0.150	0.1534	Lactic.	0.100	0.102	0.978	50.35	721.
T	" "	0.150	0.1541	"	0.150	0.154	0.974	50.35	720.
B	" "	0.300	0.3119	"	0.100	0.104	0.962	48.71	721.
T	" "	0.300	0.5152	"	0.300	0.315	0.952	48.59	720.
B	NaH_2PO_4	0.0375	0.0376	H_3PO_4	0.011	0.011	0.996	50.33	710.
"	"	0.075	0.0755	"	0.019	0.019	0.994	49.50	710.
"	"	0.120	0.1210	"	0.024	0.024	0.992	48.61	710.
"	"	0.150	0.1512	"	0.015	0.015	0.992	48.12	712.
"	"	0.300	0.3042	"	0.030	0.030	0.986	45.49	718.
"	"	0.300	0.3042	"	0.030	0.030	0.986	45.42	712.
B	KH_2PO_4	0.150	0.1516	H_3PO_4	0.015	0.015	0.990	49.16	720.
"	"	0.300	0.3055	"	0.030	0.030	0.982	46.70	720.
"	"	0.300	0.3055	"	0.030	0.030	0.982	46.30	712.
B	KHC_2O_4	0.300	0.3057	None.	0	0	0.979	49.49	718.
"	"	0.600	0.6198	"	0	0	0.968	47.19	718.

$\Delta\alpha$ = depression of CO_2 solubility caused by salts or acids in solution.

Acidified salt solution.	$\Delta\alpha$ Observed total, 0.5455 − α. (a)	$\Delta\alpha$ Due to free acid. (b)	$\Delta\alpha$ Due to salt. (a) − (b)	$\Delta\alpha$ Per mol salt. (a) − (b)	α^0	$\Delta\alpha^0$ Observed total, 0.5471 − α^0. (c)	$\Delta\alpha^0$ Due to free acid. (d)	$\Delta\alpha^0$ Due to salt. (c) − (d)	$\Delta\alpha^0$ Per mol salt. (c) − (d)
cc. CO_2 per cc. solution	cc. CO_2 per cc. solution	cc. CO_2 per cc. solution	cc. CO_2 per cc. solution	cc. CO_2 per cc. solution	cc. CO_2 per gm. H_2O	cc. CO_2 per gm. H_2O	cc. CO_2 per gm. H_2O	cc. CO_2 per gm. H_2O	cc. CO_2 per gm. H_2O
0.5300	0.0155	0.0003	0.0152	0.101	0.5327	0.0144	0.0002	0.0142	0.094
0.5276	0.0179	0.0003	0.0176	0.116	0.5302	0.0169	0.0002	0.0167	0.110
0.5116	0.0339	0.0003	0.0336	0.112	0.5157	0.0314	0.0002	0.0312	0.103
0.5113	0.0342	0.0003	0.0339	0.113	0.5154	0.0317	0.0002	0.0315	0.104
			Mean.	0.113					0.103
0.5325	0.0130	0.0003	0.0127	0.085	0.5363	0.0108	0.0002	0.0106	0.070
0.5314	0.0141	0.0003	0.0138	0.092	0.5343	0.0128	0.0002	0.0126	0.083
0.5207	0.0248	0.0003	0.0245	0.082	0.5260	0.0211	0.0002	0.0209	0.069
0.5187	0.0268	0.0003	0.0265	0.088	0.5241	0.0230	0.0002	0.0228	0.075
			Mean.	0.087					0.074
0.5214	0.0241	0.000	0.0241	0.161	0.5303	0.0168	−0.0033	0.0201	0.132
0.5220	0.0235	0.000	0.0235	0.157	0.5350	0.0121	−0.0049	0.0170	0.111
0.5023	0.0432	0.000	0.0432	0.144	0.5174	0 0297	−0.0033	0.0330	0.107
0.4980	0.0475	0.000	0.0475	0.159	0.5207	0.0264	−0.0098	0.0362	0.116
			Mean.	0.155					0.116
0.5278	0.0177	0.000	0.0117	0.118	0.5398	0.0073	−0.0033	0.0106	0.069
0.5288	0.0167	0.000	0.0167	0.111	0.5429	0.0042	−0.0049	0.0091	0.059
0.5106	0.0349	0.000	0.0349	0.116	0.5308	0.0163	−0.0033	0.0196	0.063
0.5103	0.0352	0.000	0.0352	0.117	0.5362	0.0109	−0.0098	0.0207	0.066
			Mean.	0.116					0.064
0.5360	0.0095	0.0005	0.0090	0.243	0.5381	0.0090	0.0002	0.0088	0.234
0.5272	0.0183	0.0008	0.0175	0.233	0.5302	0.0169	0.0003	0.0166	0.220
0.5175	0.0260	0.0010	0.0270	0.225	0.5217	0.0254	0.0004	0.0250	0.207
0.5109	0.0346	0.0006	0.0340	0.226	0.5151	0.0320	0.0002	0.0318	0.210
0.4791	0.0664	0.0013	0.0651	0.217	0.4864	0.0607	0.0005	0.0602	0.198
0.4823	0.0632	0.0013	0.0619	0.206	0.4891	0 0580	0.0005	0.0575	0.189
Mean of 0.15 and 0.30 M solutions.				0.218					0.201
0.5163	0.0292	0.0006	0.0286	0.291	0.5237	0.0234	0.0002	0.0232	0.153
0.4906	0.0549	0.0013	0.0536	0.179	0.4996	0.0475	0.0005	0.0470	0.154
0.4915	0.0540	0.0013	0.0527	0.179	0.5005	0.0466	0.0005	0.0461	0.154
			Mean.	0.185					0.154
0.5211	0.0244	0.000	0.0244	0.081	0.5328	0.0143	0.000	0.0143	0.046
0.4969	0.0486	0.000	0.0486	0.081	0.5134	0.0337	0.000	0.0337	0.054
			Mean.	0.081					0.050

where p represents the CO_2 pressure in mm. of mercury at the time of saturation, and $[CO_2]$ the volume per cent of CO_2 found in the solution analyzed at room temperature. V_R and $V_{38°}$ represent respectively the volumes occupied by 1 gm. of the solution at room temperature (when analyzed) and at the temperature of saturation. If a solution is cooled from a saturation temperature of 38° to a room temperature of 20° before it is

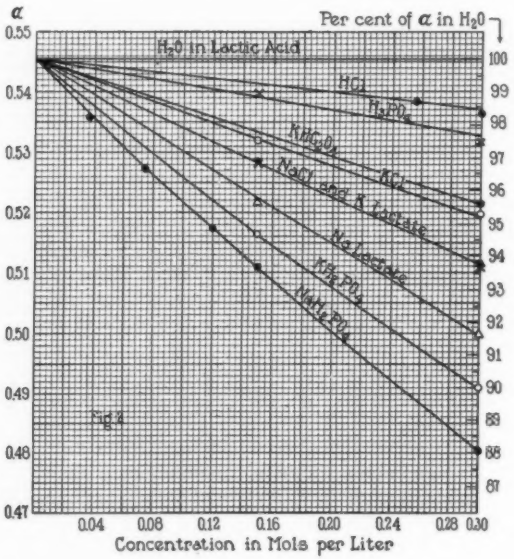

FIG. 2. Solubility of CO_2 at 38° in solutions of salts and acids. Points represent average results of analyses listed in Tables III and IV.

analyzed, it contracts by 0.5 per cent of its volume, and the volume per cent CO_2 content found by the analysis becomes 0.5 per cent higher than it was at 38°. Hence the necessity for including the factor $\frac{V_R}{V_{38°}}$ in the usual formula for calculating α. In calculating the value of $\frac{V_R}{V_{38°}}$ the coefficient of expansion of the solutions

analyzed has been assumed to be the same as that of water. At 20° the value of $\frac{V_R}{V_{38°}}$ is 0.995.

For convenience in indicating the effects of dissolved substances on the solubility of CO_2 per unit of water in the solutions, we use the symbol α^0 to indicate the cc. of CO_2, reduced to standard 0° and 760 mm., that are dissolved, under 760 mm. of CO_2 tension,

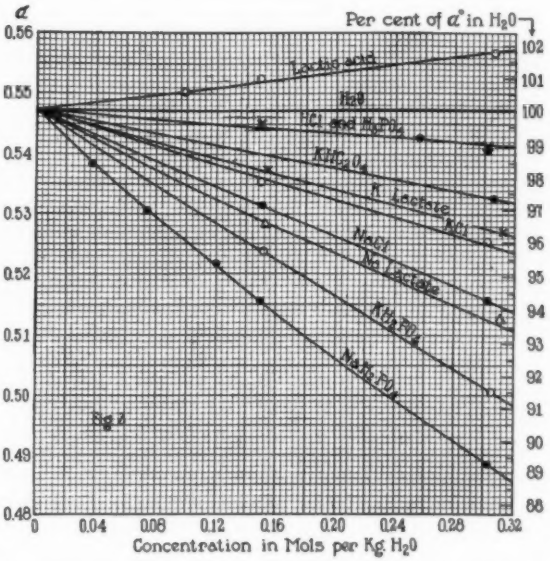

FIG. 3. Solubility of CO_2 at 38° per gram of water in solutions of salts and acids. Points represent average results of analyses listed in Tables III and IV.

per gram of water in the solution. α^0 is therefore a CO_2:H_2O ratio, and is calculated as

$$\alpha^0 = \frac{760}{p} \times \frac{[CO_2]}{100\ (H_2O)} \tag{3}$$

(H_2O) represents the grams of water per cc. of solution, the sample of solution for (H_2O) determination being measured at the same

room temperature as the sample for CO_2. Because both analyses are made thus at the same temperature, and α^0 expresses the ratio $CO_2 : H_2O$ rather than CO_2 : volume, the $\frac{V_R}{V_{38°}}$ factor does not enter into the calculation of α^0.

DETAILS AND RESULTS OF SOLUBILITY DETERMINATIONS.

Solubility of CO_2 in Water and in Solutions of Acids and Salts.—Our data on these values are given in Tables II to IV.

In Figs. 2 and 3 the solubility values are represented by curves which permit comparison of the effects of the different salts and acids on the solubility of CO_2 in aqueous solutions. The particular salts and acids studied were chosen because it was necessary to know their effects on the solubility of CO_2 in order to estimate the effects of the other constituents of cells and serum.

Water.—For the solubility coefficient of CO_2 in water at 38° our value 0.5455 is 1.7 per cent lower than the value 0.555 published in 1905 by Bohr. Bohr's value, however, was not actually determined at 38°. It was obtained by interpolation on a curve constructed from a number of α values measured by Bohr (1899) at temperatures from 0.1–61.4°. To judge from the grouping of points about the curve, the difference between Bohr's results and ours may not be outside the experimental error of his method plus the error involved in the interpolation. We have repeated six times in duplicate the determination at 38° ± 0.1°, and all six values, given in Table II, are between 0.5444 and 0.5459. Half of our saturations were by the bubbling technique and half by the tonometer technique. For the temperature 38° it appears probable that our average value 0.5455 is exact within 1 unit in the third decimal place.

Salt Solutions.—The curves of Fig. 2 indicate the presence of additive ionic effects in lowering the solubility of CO_2 in water solutions. The sodium salts regularly depress α more than do the corresponding potassium salts, and among the anions chloride, lactate, and acid phosphate rank in ascending order in their ability to depress α. Geffcken (1904, p. 285), from his solubility figures in more concentrated salt solutions, pointed out the occurrence of such ionic series, and the possibility of additive ionic effects. Our results indicate that, for the salts we have used, in the con-

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centration range below 0.3 M, the effects on the solubility of CO_2 can be calculated practically within the limit of experimental error by adding the effects of the component ions.

In order to calculate the effects of the different cations and anions on α_{CO_2} we have to make an arbitrary assumption of the magnitude of the effect of some particular ion, and use this assumed value as a starting point for estimating the relative effects of the other ions. If we assume a value for some cation, and the value is higher than the actual one, the values for all the other cations will be too high by the same margin, and the values for all the anions will be correspondingly low; but the value for any given electrolyte obtained by adding those of its ions will be correct because the positive error of the additive figure for one ion will be balanced by the negative error of the figure for the opposite ion.

We have accordingly assumed that the effect of the hydrogen ion on the solubility of CO_2 in water is zero. It appears that, in fact, its effect is relatively slight. The data in the literature (Geffcken, 1904) on the solubility of CO_2 in solutions of strong acids and their salts show that of all the inorganic cations the hydrogen ion has the smallest effect. Thus, in Fig. 2, it is obvious that HCl has much less effect in depressing α_{CO_2} than has KCl or NaCl. The effect of a 0.1 N solution of HCl is in fact hardly outside the limit of experimental error. We have accordingly assumed that the H^+ ion is without effect, and that the depression of α_{CO_2} caused by HCl is due entirely to the Cl^- ion. The "molar α depression" given in Table III for HCl was subtracted from the molar depressions of NaCl and KCl (Table IV) respectively in order to estimate the depressions of α_{CO_2} caused by Na^+ and K^+ ions per unit of molar concentration. The values for the lactate$^-$, $H_2PO_4^-$, and $HC_2O_4^-$ anions were estimated by subtraction of the Na^+ and K^+ values from the molar depressions of the respective alkali salts. The molar depressions for each ion obtained from its different salts showed some variation, partly due doubtless to experimental error, partly to failure of the ionic effects to be exactly additive. The mean value for each ion is given in Table V.

In Table VI the molar depressions of the different strong electrolytes experimentally found, from the data of Tables III and IV, are compared with the molar depressions estimated by

adding the ionic depressions from Table V. The maximum deviation between calculated and observed molar depressions of α_{CO_2} is 0.006. This corresponds for a 0.3 M salt solution, the

TABLE V.

Effects of Different Ions in Depressing Solubility of CO_2 in Water at 38°.

Figures are valid for concentrations below 0.3 M. Ions are arranged in the order of their effects on α^0.

Ion.	Depression of CO_2 solubility per unit M concentration of ion present.		Per cent by which CO_2 solubility in pure water is depressed per unit M concentration of ion present.	
	$\Delta\alpha$	$\Delta\alpha^0$	$\Delta\alpha$	$\Delta\alpha^0$
H^+	0	0	0	0
$HC_2O_4^-$	0.026	0.000	4.7	0.0
$Lactate^-$	0.066	0.018	12.1	3.3
Cl^-	0.029	0.019	5.3	3.5
K^+	0.053	0.049	10.6	9.3
Na^+	0.085	0.090	15.6	16.7
$H_2PO_4^-$	0.137	0.110	25.1	20.1

TABLE VI.

Comparison of Observed Depressions of CO_2 Solubility by Salts in 0.15 to 0.30 M Concentration with Depressions Calculated as Sum of Ionic Effects.

Salt.	Depression of CO_2 solubility per unit M concentration of salt.				Per cent depression of CO_2 solubility per unit M concentration of salt.			
	$\Delta\alpha$		$\Delta\alpha^0$		$\frac{100\,\Delta\alpha}{0.3455}$.		$\frac{100\,\Delta\alpha^0}{0.347}$.	
	Observed.	Calculated.	Observed.	Calculated.	Observed.	Calculated.	Observed.	Calculated.
HCl	0.029	0.029	0.019	0.019	5.3	5.3	3.5	3.5
NaCl	0.111	0.114	0.103	0.109	20.7	20.9	18.8	19.9
KCl	0.087	0.082	0.074	0.068	15.9	15.0	13.5	12.4
Na lactate	0.155	0.151	0.116	0.108	28.4	27.7	21.2	19.8
K "	0.116	0.119	0.064	0.067	21.3	21.8	11.7	12.2
NaH_2PO_4	0.218	0.222	0.201	0.200	40.2	40.7	36.7	36.5
KH_2PO_4	0.185	0.190	0.153	0.159	35.9	34.8	28.0	29.1
KHC_2O_4	0.081	0.081	0.050	0.050	14.8	14.8	9.1	9.1

strongest with which we worked, to a difference in α_{CO_2} of only 0.002, which is 1 part in 270, or about the experimental error.

It appears therefore that for the solutions studied of less than

0.3 M concentration, the depression of CO_2 solubility caused by the salts studied can be calculated by addition of the effects of each

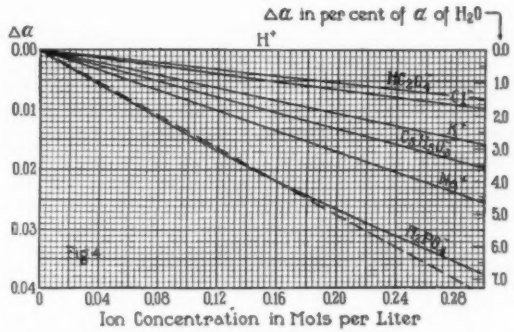

FIG. 4. Depression of Bunsen solubility coefficient by different ions. $\Delta\alpha$ = depression of α. Dash line indicates $\Delta\alpha$ for $H_2PO_4^-$ calculated from mean molar $\Delta\alpha$ when in Table V.

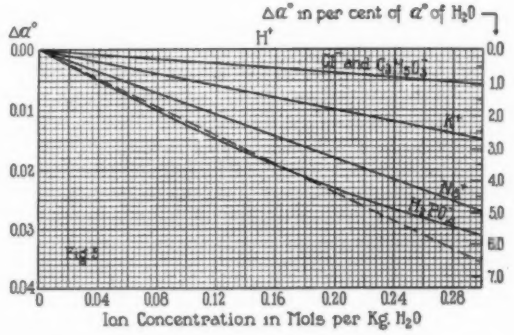

FIG. 5. Depression of CO_2 solubility per gram of water caused by different ions. $\Delta\alpha^0$ = depression of α^0. Dash line indicates $\Delta\alpha^0$ for $H_2PO_4^-$ calculated from mean molar $\Delta\alpha^0$ value in Table V.

ion, such effect being calculated by multiplying the molar depression value in Table V by the molar concentration of the ion.

The extents to which the different ions in varying concentration have been found, as above described, to depress the solubility of

CO_2 in water are shown by the curves of Figs. 4 and 5. For concentrations below 0.3 M, the effect of each ion except $H_2PO_4^-$ is a linear function of the concentration. The curves of the $H_2PO_4^-$ effects are definitely convex towards the horizontal axis. The data of Geffcken (1904) and Setschenow (1892) show a similar convexity of the curves of electrolytes in general when ranges of higher concentration are covered. Setschenow (1892) arrived empirically at the conclusion that the relative solubility, $\frac{\alpha \text{ in salt solution}}{\alpha \text{ in water}}$, varied inversely as the logarithm of the salt

TABLE VII.

Solubility of CO_2 at 38° in Normal Plasma and Serum Acidified with 0.1 Mol of Lactic Acid per Liter.

Material.	CO_2 content at 22–24°.	CO_2 tension at 38°.	H_2O content at 22–24°.	Solubilities.		Relative solubilities.		
				α	α^0	$\frac{\alpha \text{ serum}}{\alpha \text{ water}}$.	$\frac{\alpha^0 \text{ serum}}{\alpha^0 \text{ water}}$.	$\frac{\alpha^0 \text{ serum}}{\alpha^0 \text{ saline}}$.
	vol. per cent	*mm.*	*gm. per cc.*	*cc. CO_2 per cc. solution*	*cc. CO_2 per gm. H_2O*			
Ox serum	47.51	706	0.931	0.509	0.549	0.933	1.004	1.031
" "	47.90	709	0.925	0.511	0.555	0.938	1.015	1.043
Normal human serum	47.40	709	0.921	0.506	0.552	0.927	1.008	1.037
Normal human oxalate plasma	47.32	700	0.925	0.511	0.555	0.938	1.015	1.043
Average			0.9255	0.509	0.553	0.934	1.011	1.038

concentration. In our relatively dilute solutions there is no evidence of such a logarithmic relationship, except in the phosphate solutions.

Normal Serum and Plasma.

Samples of normal plasma and serum were acidified by addition of 1 volume per cent of concentrated lactic acid (sp. gr. 1.20) which is approximately enough to make a 0.1 N solution. It was sufficient to depress the pH to approximately 3.5 (determined electro-

metrically). The solubility coefficient of CO_2 in the acidified serum or plasma was determined by saturation in a rotating tonometer, as described above. The results are given in Table VII.

That the changes produced in the serum by addition of the lactic acid did not significantly alter the solubility coefficient of CO_2 appears probable from the following considerations. The concentration of lactate anions produced by replacement of the bicarbonate and protein anions of serum is 35 to 45 milli-equivalents per liter, about 25 by replacement of bicarbonate and 12 to 18 by replacement of serum protein anions (Van Slyke, Wu, and McLean, 1923). From Fig. 4 we estimate that lactate anions in 40 mM concentration depress α_{CO_2} at 38° by 0.0026, which is 0.5 per cent of the solubility in water, an amount that hardly exceeds our experimental error. The actual α_{CO_2} depression caused by addition of lactic acid to serum is presumably less than this: it is the difference between it and the depression caused by the replaced anions, chiefly HCO_3^-. Free lactic acid in amounts greater than those present has no measurable effect on the α_{CO_2} in water solution. We appear justified in considering that the solubility of CO_2 in serum is altered by less than 1 part in 200 by the added lactic acid, and that the α_{CO_2} values in Table VII apply as they stand to serum in its natural state.

The "α^0 saline" values by which the figures in the last columns of Tables VII and VIII were calculated were computed according to the following considerations. The salts in serum acidified with lactic acid consist of approximately 0.160 equivalents of total electrolyte per liter, of which nearly 90 per cent consists of sodium chloride and lactate. As shown by Fig. 3, the effects of NaCl and Na lactate on α^0, the CO_2 solubility per gram of water, are so nearly alike that below 0.16 M concentration the differences hardly exceed the experimental error. It appears therefore that we can with little error estimate the effects of the entire 0.160 equivalents of electrolyte on α^0 to be equal to that of 0.16 M NaCl. From the molar $\Delta\alpha^0$ value, 0.103, of NaCl (Table IV) we calculate the depression of α^0 by 0.16 mols of the salt per kilo of water to be 0.0165 at 38°. However, of the 0.100 mol of lactic acid added about 0.060 remains free, and from the data of Table III this concentration is sufficient to raise α^0 by 0.002 units. The total effect of the salts

and free lactic acid is therefore to depress α^0 by 0.0145. Hence if the solubility of CO_2 per gram of water in serum were affected only by the salts and the added lactic acid, the estimated α^0 would be that of water, 0.5471, reduced by 0.0145, or 0.5326. This is the value assumed for "α^0 saline" in calculating the figures for the last columns of Tables VII and VIII. The average value, 1.038, for the relative solubility ratio, $\frac{\alpha^0 \text{ in serum}}{\alpha^0 \text{ in saline}}$, indicates that the organic constituents of the four sera dissolved on the average enough CO_2 to increase the amount dissolved per gram of water to the extent of 3.8 per cent above what it would be if the organic constituents were inert as CO_2 solvents. It is difficult to estimate the probable error of this calculation, but it appears that we may be justified in stating that the organic constituents of normal serum are responsible for 3.8 ± 1.0 per cent of the CO_2 which the serum can take up by physical solution.

Effects of Lipoids and other Organic Constituents on Solubility of CO_2.

It has been shown above that normal serum and plasma dissolve about 4 per cent more CO_2 than they would if their constituents other than water and salts were inert towards this gas. Stoddard (1926–27) found a similar excess solubility of nitrogen gas in serum, and showed that it was due to the solubility of the gas in the lipoids. We find likewise that the solubility of CO_2 in excess of that attributable to the aqueous saline solution present is due at least in chief part to the solvent effect of the lipoids. This effect is not surprising in view of the fact that oils in general show solubility coefficients for CO_2 higher than that of water. The effect in plasma indicates a solvent power of the lipoids several times that of water. The two lipemic human plasmas and the lipemic serum reported in Table VIII show before extraction with ether α^0 values 10 to 12 per cent above those from Fig. 3 for NaCl solutions of the same salt contents. After extraction with ether the α^0 values were only 2 to 5 per cent above those estimated for saline solutions of similar content. The lipoids were not completely removed by ether extraction, and the excess solubility remaining in the extracted serum is attributable at least in part to the unextracted lipoids. That a part may be attributable to a slight solvent effect of the proteins for CO_2 is not excluded.

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However, in comparison with the lipoids, the proteins in the serum act as relatively inert substances. They appear neither to dissolve significant amounts of CO_2 themselves nor to affect the CO_2-dissolving power of the saline water of the plasma. Their chief effect is to decrease the volume of CO_2 dissolved per cc. of serum by displacing water. The proteins thus markedly depress the total CO_2 solubility, α, per cc. of serum, but not the solubility, α^0, per gram of serum water.

In depressing α, the proteins and salts act together, although physically in quite different ways, while the opposing influence of the lipoids is to increase α. Usually the influence of the proteins and salts preponderates; and α, the volume of CO_2 dissolved per volume of fluid, is lower in serum than in water. In our normal sera α averaged 0.510 compared with 0.546 determined by the same methods for water at 38°. In the lipemic sera encountered in some pathological conditions the α values exceed those of normal serum. Thus in Sample 3 of Table VIII the α value is 0.551. It appears that in extreme cases of lipemia, such as are met in nephrosis and severe diabetes, values of α occur which exceed even that of pure water. The low protein content usually encountered in pathologically lipemic sera assists the high fat content in raising the α value.

The organic serum substances other than proteins and lipoids do not appear to affect the solubility of CO_2 to a degree capable of measurement by our methods. The solution of serum proteins, of which the analysis is given in Sample 5 a of Table VIII, behaved quite like serum itself.

Details of Experiments Recorded in Table VIII.—In each case the plasma or serum was acidified with 1 volume per cent of lactic acid of 1.20 specific gravity. The material in Samples 1, 2, and 3 was lipemic plasma and serum obtained for us by Drs. MacKay and Möller from three patients with nephrosis. The plasmas were milky with fats, but low in proteins, in accord with the usual composition of plasmas from nephrotic subjects. A portion of each sample was extracted three times with ether, 2.5 volumes of ether to 1 volume of plasma or serum being used for each extraction. The ether was then removed by bubbling through a rapid current of air. The unextracted control sample was subjected to passage of the same air current, in order to maintain parallel treatment in conditions other than the extraction. The latter removed the visible fat. Plasma Samples 1 and 2 were intended for preliminary experiments, and unfortunately fat determinations were not performed; the entire material was used for the solubility determinations and other analyses.

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TABLE VIII.

Effect of Lipoids on Solubility of CO_2 in Plasma and Serum.

Sample of material.	Treatment before acidification.	H_2O content.	Protein content.	Total base.	Lipoids.	α	α^0	α^0 saline estimated.	Relative solubilities.		
									$\frac{\alpha \text{ serum}}{\alpha \text{ water}}$.	$\frac{\alpha^0 \text{ serum}}{\alpha^0 \text{ water}}$.	$\frac{\alpha^0 \text{ serum}}{\alpha^0 \text{ saline}}$.
		gm. per cc.	*gm. per gm. H_2O*	*eq. per kg. H_2O*	*gm. per gm. H_2O*	*cc. CO_2 per cc. serum*	*cc. CO_2 per gm. H_2O*	*cc. CO_2 per gm. H_2O*			
1. Human lipemic oxalate plasma (nephrosis).	a. None.	0.936	0.035	0.286		0.535	0.574	0.524	0.981	1.049	1.096
	b. Extracted with ether.	0.944	0.037	0.292		0.500	0.532	0.524	0.917	0.973	1.015
2. Human lipemic oxalate plasma (nephrosis).	a. None.	0.934	0.037	0.260		0.542	0.582	0.526	0.993	1.064	1.106
	b. Extracted with ether.	0.946	0.035	0.259		0.510	0.542	0.526	0.935	0.932	1.030
3. Human lipemic serum (nephrosis).	a. None.	0.934	0.039	0.149	0.0316	0.551	0.592	0.531	1.010	1.082	1.115
	b. Extracted with ether.	0.946	0.038	0.152	0.0048	0.520	0.552	0.531	0.953	1.009	1.039
4. Horse serum free from visible fat.	a. None.	0.921	(0.070)	(0.160)	0.0031	0.522	0.569	0.531	0.957	1.040	1.071
	b. Extracted with ether.	0.930	(0.070)	(0.160)	0.0027	0.517	0.559	0.531	0.947	1.022	1.053
5. Solution of serum proteins in 0.15 M NaCl.	a. None.	0.963	0.026	0.156	0.0011	0.530	0.551	0.531	0.972	1.007	1.038
	b. Added olive oil.	0.928	0.025	0.155	0.0356	0.690	0.745	0.531	1.265	1.362	1.403

Figures for total base and protein content of normal horse serum, in parentheses, were not determined by analysis of the serum sample used for this experiment. They are the usual values found previously for blood from the same horse.

Sample 4 was horse serum low in fat content. It was entirely clear, with no visible fat emulsion at all. The content of the invisible lipoids was but slightly affected by ether extraction.

Sample 5 was a solution of serum proteins. The latter were prepared by diluting 400 cc. of serum to 1600 cc. and precipitating by saturation with ammonium sulfate and CO_2. The precipitate was separated by centrifugation, was extracted with ether, redissolved in water, and again precipitated with ammonium sulfate. It was dialyzed in closed collodion sacs in the presence of toluene until free of sulfate. The mixture in the sacs was then brought to 250 cc. volume with sufficient NaCl to make the concentration 0.15 M. Under the influence of the salt the proteins readily redissolved.

One portion of 80 cc. of the solution was acidified with 0.80 cc. of concentrated lactic acid and used for the analyses given for Sample 5 a in Table VIII. Another portion of 65 cc. was mixed with 7 cc. of a 1 : 1 solution of olive oil and petroleum ether. A rapid stream of air was passed through the mixture, simultaneously removing the petroleum ether and emulsifying the oil. A better emulsification was obtained thus than without the initial presence of the petroleum ether. The emulsion thus obtained was acidified with 0.68 cc. of concentrated lactic acid and used for the analyses of Sample 5 b in Table VIII.

Solubility of CO_2 in Diluted Cell Solutions at 38°.

Because of the viscosity of acidified cells, it was necessary, as mentioned above, to use dilute solutions of them, and estimate the solubility in undiluted cells by extrapolation. The cells in weighed amounts were diluted to measured volumes with water, to which had been added amounts of hydrochloric, lactic, or phosphoric acid proportional to the concentration of cells in the first solution, and sufficient to reduce the pH below 3.5. The volume of cells taken in each case was calculated from the weight and the specific gravity. The results of the solubility determination are given in Tables IX and X.

Unlike serum, cells do not permit us to neglect the correction for the anions formed from the added acid. The effects on the quantitatively important electrolytes of the cells of adding an acid, HA, in sufficient amount to depress the pH to 3.0 to 3.5, may be symbolized as follows:

(*a*) $B^+Cl^- \rightarrow B^+Cl^-$

(*b*) $B^+HCO_3^- \rightarrow B^+A^- + H_2CO_3$

(*c*) $B^+Hb^- \rightarrow B^+A^- + Hb^+A^-$

of the serum sample used for this experiment. They are the usual values found previously for blood from the same horse.

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Of these effects only the last (Reaction c) can be important in affecting CO_2 solubility. The chlorides (Reaction *a*) are practically uneffected. The $BHCO_3$ concentration is so small in cells, about 15 mM, that the effect of HCO_3^- replacement by either lactate, $H_2PO_4^-$, or Cl^- anions must be quantitatively negligible. The concentration of lactate$^-$ or $H_2PO_4^-$, anions formed by Reaction *c*, however, is enough to affect measurably the CO_2 solubility. We have corrected for the depressant effect of the Cl^-, lactate$^-$, and $H_2PO_4^-$ anions, formed by addition of the respective acids, by calculating the concentrations of the anions thus formed, and adding to the observed α and α^0 values the $\Delta\alpha$ and $\Delta\alpha^0$ corrections estimated from Figs. 4 and 5. This correction, as seen in Fig. 6, has reduced nearly to the limit of experimental error the differences between results obtained from cells acidified with the different acids.

The correction embodies the assumption that changing the charge of the Hb molecules from negative to positive, as indicated by Reaction *c*, does not alter their influence on α_{CO_2}. This assumption cannot be proved by experiment, for α_{CO_2} cannot be determined in solutions of sufficient alkalinity to permit the existence of BHb, and hence the estimation of the effect of Hb^-. That a considerable change in the electric charge on the Hb molecule does not, however, measurably alter the effect on α_{CO_2} is shown by the agreement between results obtained with cell solutions acidified with lactic acid to pH 3.5 and solutions acidified to pH 2.6 with phosphoric acid.

In the cell solutions to which HCl was added the increase in the Cl anions was assumed to be equal to the HCl added, since dissociation is fairly complete of either the free acid or its salts.

The concentrations of the lactate and acid phosphate anions in the cell solutions were calculated, from the amount of each acid added and the pH of the solution, by means of the familiar Henderson equation for mixtures of weak acids and their salts. The equation may be written as

$$[BA] = \frac{C}{1 + \frac{H^+}{K'}} \tag{4}$$

where C represents the total concentration of buffer, partly in the form of the salt BA, partly in the form of the free acid HA. The concentration of anions has been estimated, on the assumption of complete dissociation of the salt BA, to be equal to [BA]. This same assumption has been made in estimating the effects of the lactate$^-$ and $H_2PO_4^-$ anions on the solubility of CO_2 (Table V and Figs. 4 and 5); whatever error there may be in this assumption is neutralized, in so far as significant error in the present application is concerned, by using the same assumption in the previous determination of the ionic α_{CO_2} depressions.

To determine the factor $\frac{1}{1 + \frac{H^+}{K'}}$ by which the added acid, C, must be

multiplied to estimate the concentration of its anions, it is necessary to know H^+ and K', the apparent dissociation constant of the acid in the buffer mixture. The H^+ values were determined with the hydrogen electrode.

The K' value for the dissociation of the first hydrogen of H_3PO_4 has been estimated by Van Slyke (1922) from hydrogen electrode titrations of Clark and Lubs (1916) to be 1×10^{-2}. The pH of the cell solutions to which H_3PO_4 was added was reduced uniformly to 2.6, corresponding to an H^+ value of 2.5×10^{-3}. Substituting these two values for K' and H^+ in Equation 4 we calculate that 80 per cent of the added H_3PO_4 was converted into the ionized salt, BH_2PO_4.

For lactic acid-lactate mixtures the K' value appears not to have been determined. We have accordingly made an approximate estimation of it at 38° as follows: Equal volumes of 0.2 N lactic acid and 0.1 N sodium hydroxide were mixed. Under these conditions [BA] = [HA] and $H^+ = K'$ (*e.g.* see Van Slyke, 1922). The pH of the solution was determined with the hydrogen electrode, the pH of 0.1 N HCl being taken as 1.08 to standardize the cell. The lactate mixture showed a pH of 3.74, indicating a H^+ activity of 1.8×10^{-4}. This was therefore taken as the K' value for the lactate mixtures in our solutions. It agrees quite closely with the dissociation constant 1.4×10^{-4} found by Ostwald (1889) with his conductivity method.

In lactic acid-cell solutions, Samples 1 a, 2 a, and 3 a (Tables IX and X), the amounts of lactic acid added were proportional to the concentration of cell buffers present, and the pH was uniformly depressed to 3.5, indicating a H^+ value of 3.16×10^{-4}. From this H^+ and the above K' we calculate by Equation 4 that 31 per cent of the added acid was converted into ionized lactate salts. In lactate-cell solutions, Samples 2 a, 2 b, 2 c, 3 a, and 3 b, the proportion of lactic acid per unit of cell concentration was not constant, and the pH was not determined (these were earlier experiments). The proportion of lactic acid per unit of cell concentration, was, however, of a similar order of magnitude, and we have assumed the same pH in calculating the amount of lactate salts formed and the resultant corrections to α and α^0, enclosing the values thus calculated in parentheses to indicate their approximate nature. The solubility corrections due to lactate are so small (0.0026 to α and 0.0012 to α^0) that the error involved in thus approximating the corrections can hardly affect the solubility coefficients by as much as 0.001.

The observed CO_2 solubilities in the cell solutions have been corrected in Tables IX and X, as there indicated, for the added effects of the anions of the added acids and of those portions of the

TABLE IX.

Solubility Coefficients of CO_2 in Acidified Aqueous Solutions of Ox Cells.

Sample No.	Concentration of cells.	H_2O content of solution at room temperature.	Amount of acid added.	Salts formed from added acid (estimated from pH).	Amount of added acid remaining free (estimated from pH).	$\Delta\alpha$ due to salts of added acid.	$\Delta\alpha$ due to free acid.	Total α change due to added acid.	α observed.	α corrected for effect of added acid.
				Lactic acid added.						
	cc. per 100 cc. solution	*gm. per cc. solution*	*mols per l. solution*	*mols per l. solution*	*mols per l. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*
1 a	10.2	0.970	0.084	0.034	0.050	0.0022	0.000	−0.0022	0.536	0.538
b	20.6	0.940	0.180	0.072	0.128	0.0047	0.000	−0.0047	0.527	0.532
c	24.8	0.928	0.270	0.108	0.162	0.0072	0.000	−0.0072	0.520	0.527
2 a	48	0.981	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.536	0.539
b	86	0.970	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.531	0.534
c	13.4	0.955	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.527	0.530
3 a	5.1	0.983	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.537	0.540
b	10.2	0.967	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.535	0.538
				Phosphoric acid added.						
4 a	10.0	0.972	0.078	0.036	0.012	0.0060	0.0006	−0.0066	0.529	0.536
b	19.2	0.948	0.096	0.072	0.027	0.0105	0.0011	−0.0116	0.516	0.528
c	25.6	0.930	0.125	0.095	0.030	0.0135	0.0014	−0.0149	0.503	0.518
				Hydrochloric acid added.						
5 a	10.3	0.971	0.050	0.050	0.00	0.0015	0.000	−0.0015	0.531	0.533
b	20.4	0.947	0.100	0.100	0.00	0.0029	0.000	−0.0029	0.526	0.529
Extrapolated α for 100 per cent cells (Fig. 6).....										0.45

$\Delta\alpha$ indicates depression of α. Figures in parentheses are calculated from estimated pH 3.5, which was not determined in these cases.

TABLE X.

Solubility of CO_2 per Gram of Water in Acidified Aqueous Solution of Ox Cells.

Sample No.	Concentration of cells.	H_2O content of solution at room temperature.	Amount of acid added.	Salts formed from added acid (estimated from pH).	Amount of added acid remaining free (estimated from pH).	$\Delta\alpha$ due to salts of added acid.	$\Delta\alpha$ due to free acid.	Total α change due to added acid.	α observed.	α corrected for effect of added acid.
				Lactic acid added.						
	cc. per 100 cc. solution	*gm. per cc. solution*	*mols per l. solution*	*mols per l. solution*	*mols per l. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*	*cc. CO_2 per cc. solution*
1 a	10.2	0.970	0.084	0.034	0.050	0.0022	0.000	−0.0022	0.536	0.538
b	20.6	0.940	0.180	0.072	0.128	0.0047	0.000	−0.0047	0.527	0.532
c	24.8	0.928	0.270	0.108	0.162	0.0072	0.000	−0.0072	0.520	0.527
2 a	48	0.981	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.536	0.539
b	86	0.970	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.531	0.534
c	13.4	0.955	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.527	0.530
3 a	5.1	0.983	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.537	0.540
b	10.2	0.967	0.100	(0.040)	(0.060)	(0.0026)	0.000	(−0.0026)	0.535	0.538
				Phosphoric acid added.						
4 a	10.0	0.972	0.078	0.036	0.012	0.0060	0.0006	−0.0066	0.529	0.536
b	19.2	0.948	0.096	0.072	0.027	0.0105	0.0011	−0.0116	0.516	0.528
c	25.6	0.930	0.125	0.095	0.030	0.0135	0.0014	−0.0149	0.503	0.518
				Hydrochloric acid added.						
5 a	10.3	0.971	0.050	0.050	0.00	0.0015	0.000	−0.0015	0.531	0.533
b	20.4	0.947	0.100	0.100	0.00	0.0029	0.000	−0.0029	0.526	0.529
Extrapolated α for 100 per cent cells (Fig. 6).....										0.45

$\Delta\alpha$ indicates depression of α. Figures in parentheses are calculated from estimated pH 3.5, which was not determined in these cases.

$\Delta\alpha$ indicates depression of α. Figures in parentheses are calculated from estimated pH 3.5, which was not determined in these cases.

TABLE X.

Solubility of CO_2 per Gram of Water in Acidified Aqueous Solution of Ox Cells.

Sample No.	Concentration of cells.	H_2O content of solution at room temperature.	Amount of acid added.	Salts formed from added acid (estimated from pH).	Amount of acid remaining free (estimated from pH).	$\Delta\alpha^0$ due to anions of salts of added acid.	$\Delta\alpha^0$ due to free acid.	Total α change due to added acids.	α^0 observed.	α^0 corrected for effect of added acid.
Lactic acid added.										
	cc. per 100 cc. solution	*gm. per cc. solution*	*mols per kg. H_2O*	*mols per kg. H_2O*	*mols per kg. H_2O*	*cc. CO_2 per gm. H_2O*	*cc. CO_2 per gm. H_2O*	*cc. CO_2 per gm. H_2O*	*cc. CO_2 per gm. H_2O*	*cc. CO_2 per gm. H_2O*
1 a	10.2	0.970	0.088	0.035	0.053	0.0006	−0.0017	+0.0013	0.556	0.555
b	20.6	0.940	0.192	0.076	0.116	0.0014	−0.0037	+0.0023	0.563	0.561
c	24.8	0.928	0.291	0.117	0.174	0.0021	−0.0056	+0.0034	0.563	0.560
	100.0									
2 a	4.8	0.981	0.102	(0.040)	(0.061)	(0.0007)	(−0.0019)	(+0.0012)	0.549	0.548
b	8.6	0.970	0.103	(0.041)	(0.062)	(0.0007)	(−0.0019)	(+0.0012)	0.549	0.548
c	13.4	0.955	0.105	(0.042)	(0.063)	(0.0007)	(−0.0019)	(+0.0013)	0.554	0.553
	100.0									
3 a	5.1	0.983	0.102	(0.041)	(0.061)	(0.0007)	(−0.0019)	(+0.0012)	0.548	0.547
b	10.2	0.967	0.103	(0.041)	(0.062)	(0.0007)	(−0.0019)	(+0.0012)	0.555	0.554
Phosphoric acid added.										
4 a	10.0	0.972	0.049	0.038	0.011	0.0048	0.0002	−0.0050	0.546	0.551
b	19.2	0.948	0.101	0.077	0.024	0.0098	0.0004	−0.0102	0.547	0.557
c	25.6	0.930	0.134	0.102	0.032	0.0130	0.0006	−0.0136	0.543	0.557
Hydrochloric acid added.										
5 a	10.3	0.971	0.052	0.050		0.0010		−0.0010	0.549	0.550
b	20.4	0.947	0.106	0.104		0.0020		−0.0020	0.558	0.560
Extrapolated α^0 for 100 per cent cells (Fig. 6).....										0.60

$\Delta\alpha^0$ indicates depression of α^0. Figures in parentheses are calculated from estimated pH 3.5, which was not determined in these cases.

added acids remaining free. The solubility values thus corrected are given in the last columns of Tables IX and X.

From Fig. 6 it is evident that the corrected CO_2 solubility values change in direct linear proportion to the concentration of cell contents present in the solutions.

The solubility per cc. of solution, represented in the lower curve, decreases as more cells are added. The total effect of the cell

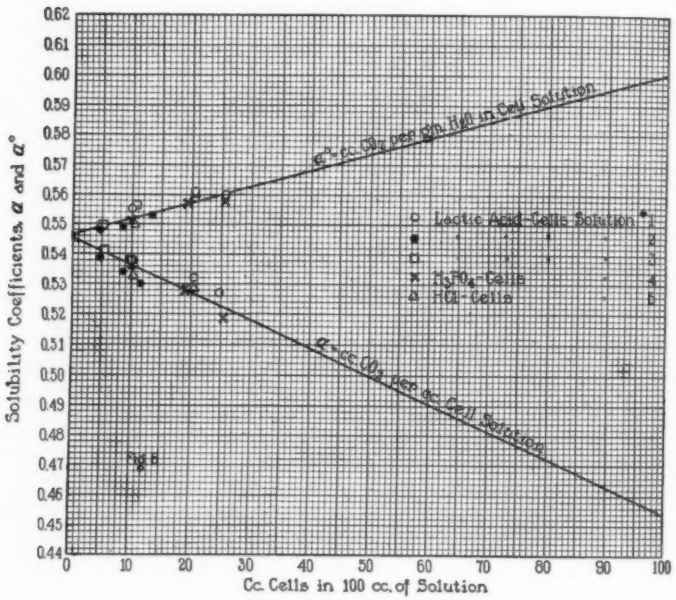

FIG. 6. Solubility of CO_2 at 38° in solutions of ox blood cells in water.

constituents is therefore to depress the solvent power per unit volume of solution.

However, the rising slope of the upper curve indicates that cell contents, like serum contents, increase the amount of CO_2 dissolved *per gram of water* in the solutions. Some organic constituent or constituents of the cells therefore act as solvents for CO_2, and their effect on the amount dissolved per gram of

water more than offsets the depressant effect of the mineral salts present. Whether in the cells, as in serum, the lipoids are responsible for the CO_2 dissolved by the organic constituents, or whether the proteins also act to an appreciable extent as CO_2 solvents, we have not been able to settle.

In order to estimate the solubilities in the undiluted cells we have extended the straight lines, representing α and α^0 in Fig. 6, until they indicate at 100 the extrapolated coefficients for the cells themselves. Such extrapolation involves the assumption that the direct linear relationship, between the concentration of cell constituents present and their effect on the solubility of CO_2, found to hold for solutions containing up to 25 per cent by volume of cells, holds also for the remaining range up to 100 per cent. It has been

TABLE XI.

Analyses of Ox Cells Used for Solubility Experiments Reported in Tables IX and X.

Sample No.	H_2O content of cells.	K content.	Na content.	Chloride content.	Fat content (Rôse-Gottlieb).	Protein content (N × 5.78).
	kg. per l.	*m.-eq. per l.*	*m.-eq. per l.*	*m.-eq. per l.*	*gm. per l.*	*gm. per l.*
1	0.718	27.3	81.6	65.8	1.90	287
2	0.669	29.9	90.9	72.1	1.22	306
3	0.716	30.1	91.7	72.2	2.72	291
4	0.743	34.0	102.1	81.8	1.17	304
5	0.749	32.0	97.0	56.6	0.84	280

impossible to prove this assumption, because of the viscosity of the more concentrated cell solutions. There is however, no reason to expect significant deviation from the linear relationship. The electrolyte content, indicated by the total base, reaches in cells only about 170 milli-equivalents per kilo of water present (Van Slyke, Wu, and McLean, 1923), and the curves of Figs. 2 and 3 show hardly perceptible deviations from straight lines below this concentration. That the effect of the non-electrolytes, in the relatively small molar concentration present, would be other than linear, appears improbable.

The extrapolation of the upper curve of Fig. 6 indicates that cells saturated with CO_2 at atmospheric pressure and 38° dissolve 0.60 cc. of CO_2, measured under standard conditions, per gram of water present. The probable error of the extrapolation appears

to be about 0.01 cc. The ordinary Bunsen solubility coefficient, plotted in the lower curve of Fig. 6, indicates by extrapolation an α value for the ox cells used of 0.45 cc. of CO_2 per cc. of cells. The ox cells contained varying proportions of water (see Table XI). Human cells average about 0.73 gm. of water per cc. (unpublished data) and, with the same solvent power as ox cells per gram of water, would have a Bunsen solubility coefficient of 0.44.

TABLE XII.

Solubility of CO_2 in Cell Solutions and Serum Saturated at Low and High CO_2 Pressures.

Solution analyzed.	CO_2 tension at 38°.	CO_2 content at 22.0–22.5°.	α	α^b
	mm.	*vol. per cent*	*cc. CO_2 per cc. solution*	*cc. CO_2 per gm. H_2O*
50 cc. horse cells + 150 cc. H_2O + 20 cc. 1.0 N HCl.				
H_2O = 0.928 gm. per cc. at 22.5°.	709.7	48.50	0.517	0.560
Protein = 0.085 gm. per cc.		48.61	0.518	0.561
Total base = 34.6 m.-eq. per kg. H_2O.	60.2	4.14	0.520	0.563
pH = 3.7.		4.12	0.517	0.561
100 cc. horse serum + 10 cc. 1.0 N HCl.	709	49.15	0.524	0.561
H_2O = 0.939 gm. per cc. at 25°.		49.45	0.527	0.564
pH = 3.2.	41.9	2.908	0.525	0.562
		2.900	0.523	0.560
	42.4	2.900	0.517	0.554
		2.925	0.522	0.559

Solubility of CO_2 in Serum and Cells at Low CO_2 Tensions.

Conant and Scott (1926)[3] recently demonstrated that when a solution of hemoglobin was equilibrated with nitrogen gas at different partial pressures, the amount absorbed per gram of water exceeded the amount absorbed by water in the absence of other substances. The excess of absorbed N_2 taken up by the hemoglobin did not, in its variations with varying N_2 tension, follow Henry's law. It followed the Freundlich adsorption equation:

(5) $$\text{Amount adsorbed} = a\,p^b$$

where p is the partial pressure of gas, and a and b are constants for a given solution, the usual value of b being 0.5 or less.

If significant amounts of the CO_2 were held by adsorption on the colloid particles or molecules one would expect to find higher solubility coefficients at low than at high tensions, as did Conant and Scott in the case of nitrogen. We therefore determined the solubility of CO_2 in acidified serum and cell solutions at CO_2 pressures of 40 to 50 mm. as well as at 1 atmosphere. The results given in Table XII show that at the lower CO_2 tensions the same solubility coefficient was obtained as at 1 atmosphere. Within the limits of CO_2 pressures under which our experiments were carried out, CO_2 is dissolved by both plasma and cells in accordance with Henry's law. There is no evidence of deviation from it such as was found by Conant and Scott in the case of nitrogen gas.

Effect of Change in α_{CO_2} on the Calculation of Serum pH by Hasselbalch's Equation.

From the following considerations it is evident that changing the value of α from Bohr's 0.541 to our 0.510 necessitates *no revision* of the serum pH values in the literature that have been calculated by Hasselbalch's equation, with the Bohr α.

The concentration of "physically dissolved CO_2," symbolized as $[H_2CO_3]$, is estimated, by definition[1] of the Bunsen solubility coefficient, α, as

$$(6)\quad \left.\begin{array}{l}[H_2CO_3] \text{ in cc. } CO_2 \\ \text{dissolved per cc.} \\ \text{of solution}\end{array}\right\} = \alpha \times (\text{atmospheres } CO_2 \text{ pressure})$$

$$= \alpha \times \frac{\text{mm. } CO_2 \text{ pressure}}{760}$$

$$= \frac{\alpha p}{760}$$

where p expresses CO_2 pressure in mm. of mercury. Hence

$$(7)\quad \left.\begin{array}{l}[H_2CO_3] \text{ in cc. } CO_2 \\ \text{dissolved per liter} \\ \text{of solution}\end{array}\right\} = 1000\,\frac{\alpha p}{760} = \frac{\alpha p}{0.76}$$

According to Guye and Pintza (1908) 1 mg. molecule of CO_2 gas at 0°, 760 mm., occupies 22.26 cc. of volume. Hence the concentration in mM per liter is calculated from the concentration in cc. per liter by dividing the latter by 22.26.

$$(8) \qquad [H_2CO_3] \text{ in mM per liter} = \frac{\alpha\, p}{22.26 \times 0.76} = 0.0591\ \alpha\, p$$

Hasselbalch's equation may be written

$$(9) \qquad \begin{aligned} pH &= pK' + \log [BHCO_3] - \log [H_2CO_3] \\ &= pK' + \log [BHCO_3] - \log (0.0591\ \alpha\ p) \\ &= pK' + \log [BHCO_3] - \log 0.0591 - \log \alpha - \log p \end{aligned}$$

It is obvious that any change in α affects pH to the same extent that it affects log α, and in the opposite direction. Increasing log α by 0.1 will decrease pH by 0.1, and *vice versa*, if the same pK′ is used.

Hence decreasing α from 0.541 to 0.510 would increase the pH, calculated by Hasselbalch's equation, by 0.026, which is the difference between log 0.541 and log 0.510.

However, if α were thus changed, one would have to revise the pK′ value used, by recalculating it from the original values of pH, p, and $[BHCO_3]$, from which the pK′ values in past use have been calculated. pK′ is calculated from experimentally determined pH, $[BHCO_3]$, and p, as:

$$(10) \qquad pK' = pH - \log [BHCO_3] + \log 0.0591 + \log \alpha + \log p$$

Hence any increase in log α would cause an equal increase in the pK′ estimated from identical data. The revision of pK′ would neutralize the revision of α, and determinations of pH from $[BHCO_3]$ and p values with *both* α and pK′ thus corrected would, by the Hasselbalch equation, yield the same pH values as with the old constants.

There would, it is true, be a slight effect on the calculated pH, when $[BHCO_3]$ is determined in the manner usually employed. $[BHCO_3]$ has usually been estimated as the difference between the total CO_2 in solution, found by analysis, and H_2CO_3, calculated from p and α.

$$(11) \qquad \begin{aligned} [BHCO_3] &= [\text{total } CO_2] - [H_2CO_3] \\ &= [\text{total } CO_2] - 0.0591\ \alpha\ p \end{aligned}$$

Hence any change in α does affect $[BHCO_3]$. However, at physiological blood pH ranges, $[H_2CO_3]$ is only about one-twentieth as great as $[BHCO_3]$. Consequently such change in α as we are concerned with causes only a negligible percentage change in $[BHCO_3]$, and a correspondingly negligible effect on the calculated pH. For example, at pH 7.4, with $[BHCO_3]$ 20-fold as great as H_2CO_3 a change of α from 0.541 to 0.510, lowering α by 5.7 per cent, would affect $[BHCO_3]$ by only $\frac{5.7}{20} = 0.28$ per cent, and the resultant alteration in calculated pH would be only 0.001, a change within the limits of error of present measurements.

It is therefore obvious that our revision of α_{CO_2} for blood serum involves no necessity for recalculating serum pH values in the literature that have been calculated by Hasselbalch's equation.

If, on the other hand, one changes from the use of Bohr's α, 0.541, to our lower α, 0.510, one must make a corresponding change in pK′, lowering it by 0.026 units. Otherwise the pH values calculated with our α would be 0.026 units too high.

SUMMARY.

The measurable deviations of the solubility of CO_2 in serum from its solubility in water have been found due to the following factors: *salts*, depressing the solubility about 3 per cent by lowering the solvent power of the water for CO_2, and to a lesser extent by displacing a small amount of water; *proteins*, depressing the solubility of CO_2 per cc. of serum several per cent by displacing water; *lipoids*, raising the solubility (by about 4 per cent in normal serum) because of their own high solvent power for CO_2. The combined effect is to reduce the solubility in normal serum to 93 to 94 per cent of its solubility in water. But in lipemic serum the solubility may exceed that in water.

The values for blood cells were obtained by extrapolation from determinations on aqueous solutions of the cells. Hence the coefficients for cells are not significant to more than the second place.

The same solubility coefficient was found for serum whether saturated at 1 or $\frac{1}{7}$ atmosphere of CO_2 pressure. Similar independence of the pressure was found for the CO_2 solubility coefficient of a solution of red blood cells. There was no evidence

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of deviation from Henry's law, such as might have been expected if any of the colloids of either serum or cells took up significant amounts of CO_2 by adsorption.

The following solubility coefficients for CO_2 in acidified solutions at 38° have been found.

	Water.	Normal human serum, average.	Lipemic human serum, maximum.	Ox blood cells, average.
α = cc. CO_2, measured under standard conditions, dissolved per cc. fluid under 760 mm. CO_2 pressure	0.5455 ±0.001	0.510	0.552	0.44*
Relative α, water α being 100	100.0	93.4	101.2	79
α^0 = cc. CO_2, measured under standard conditions, dissolved per gm. water in solution	0.5471 ±0.001	0.553	0.592	0.60
Relative α^0, water α^0 being 100	100.0	101.4	108.2	110
Factor $\frac{\alpha}{7.6}$, for formula, $[H_2CO_3]$ in vol. per cent $CO_2 = \frac{\alpha}{7.6} \times p$	0.0718	0.0671	0.0726	0.0566
Factor $\frac{\alpha}{22.26 \times 0.76}$, for formula, $[H_2CO_3]$ in mM per l. $= \frac{\alpha}{22.26 \times 0.76} \times p$	0.03222	0.0301	0.0326	0.0254
Factor $\frac{\alpha^0}{22.26 \times 0.76}$, for formula, $[H_2CO_3]$ in mM per kg. $H_2O = \frac{\alpha^0}{22.26 \times 0.76} \times p$.	0.03232	0.0327	0.0350	0.0354

* For cells with 0.73 gm. H_2O per cc.

In water solutions of the chlorides, lactates, and acid phosphates of potassium and sodium, the depressions of α and α^0 per mol of electrolyte have been found to be additive functions of the depressions due to the individual ions. In descending order of their effect on α^0 the ions rank as follows: $H_2PO_4^- > Na^+ > K^+ > Cl^- >$ lactate$^- > HC_2O_4^- > H^+$.

Revision of the solubility coefficient of serum from the α value 0.541, estimated by Bohr (1905) to the value 0.510 found by us, does not necessitate revision of the serum pH values in the literature that have been calculated by Hasselbalch's equation. The lowering of α is compensated by the lowering of 0.026 units which occurs in the pK′ of Hasselbalch's equation, when the pK′ is recalculated from the same experimental data with our lower α value.

BIBLIOGRAPHY.

Austin, J. H., Cullen, G. E., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, liv, 121.

Bohr, C., *Skand. Arch. Physiol.*, 1905, xvii, 104.

Bohr, C., *Wied. Ann. Physik u. Chem.*, 1891, xliv, 44; 1897, lxii, 644; 1899, lxviii, 500.

Bohr, C., and Bock, J., *Wied. Ann. Physik u. Chem.*, 1891, [2] xliv, 318.

Buytendyk, F. J., Brinkman, R., and Mook, H. W., *Biochem. J.*, 1927, xxi, 576.

Clark, W. M., and Lubs, H. A., *J. Biol. Chem.*, 1916, xxv, 479.

Conant, J. B., and Scott, N. D., *J. Biol. Chem.*, 1926, lxviii, 107.

Fahr, G., *J. Physiol.*, 1912, xliii, 417.

Geffcken, G., *Z. physik. Chem.*, 1904, xlix, 271.

Guye, P. A., and Pintza, A., *Mem. Soc. Physique et Hist. Nat. Genève*, 1908, xxxv, 569.

Henderson, Y., *J. Biol. Chem.*, 1918, xxxiii, 31.

Just, G., *Z. physik. Chem.*, 1901, xxxvii, 342.

Ostwald, W., *Z. physik. Chem.*, 1889, iii, 173.

O'Brien, H. R., and Parker, W. L., *J. Biol. Chem.*, 1922, l, 289.

Schoen, R., *Z. physiol. Chem.*, 1923, cxxvii, 243.

Setschenow, J. M., *Ann. chim. et physique*, 1892, xxv, 226.

Siebeck, R., *Skand. Arch. Physiol.*, 1909, xxi, 268.

Stoddard, J. L., *J. Biol. Chem.*, 1926–27, lxxi, 629.

Van Slyke, D. D., *J. Biol. Chem.*, 1922, lii, 551; 1923–24, lviii, 523.

Van Slyke, D. D., Hiller, A., and Berthelsen, K. C., *J. Biol. Chem.*, 1927, lxxiv, 659.

Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523.

Van Slyke, D. D., and Sendroy, J., Jr., *J. Biol. Chem.*, 1927, lxxiii, 127.

Van Slyke, D. D., Wu, H., and McLean, F. C., *J. Biol. Chem.*, 1923, lvi, 765.

STUDIES OF GAS AND ELECTROLYTE EQUILIBRIA IN BLOOD.

XI. THE SOLUBILITY OF HYDROGEN AT 38° IN BLOOD SERUM AND CELLS.

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Fahr's (1911–12) determinations of the solubility of hydrogen at 29.7° appear to be the only ones in the literature in which the solvent powers of blood cells and serum for this gas have been determined. Hydrogen is occasionally used as an inert gas in physiological work (*e.g.* the lung volume determination of Van Slyke and Binger (1923), Lundsgaard and Schierbeck (1923)). A knowledge of the solubility of the gas in blood serum and cells at body temperature seems desirable. Hence we present the accompanying data, which were originally obtained for a specific application.

The serum and cells used were obtained by centrifugation of defibrinated ox blood.

The saturation, with hydrogen gas from a metal tank, was performed in a rotating double-chambered saturating vessel according to the "First Saturation Method" of Austin *et al.* (1922). The hydrogen atmosphere was renewed twice during the saturation, which was prolonged to an hour.

The H_2 content of the liquid phase was determined in a Van Slyke-Neill (1924) manometric apparatus with a chamber of 100 cc. capacity and a calibration mark at 1.000 cc. for measurement of the volume of extracted H_2 at the time of its pressure reading. Samples of 15 cc. each of saturated cells or serum were transferred from the smaller chamber of the saturating vessel to the manometric apparatus by means of the special pipette described in section XII of Van Slyke and Neill's paper (1924). The H_2 gas

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was extracted from the fluid by 3 minutes shaking in the evacuated chamber. To remove CO_2 and possible traces of O_2 from the extracted gas, the latter was transferred to the special Hempel pipette described by Van Slyke and Hiller ((1928), see Fig. 2), where the gas was shaken with alkaline sodium hydrosulfite solution.

Before the H_2 was returned to the chamber for measurement the serum or cells were washed out of the chamber and replaced by 15 cc. of water, so that the gas volume at the final reading could be taken over a transparent fluid with a perfectly sharp meniscus. The air in the water was extracted and ejected, and the manometer reading p_0 was taken with the meniscus of the solution at the 1 cc. mark in the gas-free chamber.

The hydrogen gas from the Hempel pipette was then returned to the chamber, and the manometer reading p_1 was taken with the meniscus again at the 1 cc. mark. The hydrogen content of the analyzed fluid was calculated as

$$\text{Volume per cent } H_2 = (p_1 - p_0) \times \text{factor}$$

The factor in the above equation was computed by the formula given on p. 542 of Van Slyke and Neill's paper. The α'_{H_2} values used in the formula were from Winkler's data in the Landolt-Börnstein "Tabellen." The i factor was taken as 1.000.

The hydrogen gas from 15 cc. of saturated cells or serum was sufficient to give at 1 cc. volume a pressure of approximately 170 mm., duplicate determinations at the same temperature being constant to within 1 mm.

The H_2 with which the serum had come to equilibrium contained slight amounts of CO_2; when cells had been saturated there was also a little oxygen extracted from them, and a slight amount was present as impurity in the hydrogen used. The amounts of these gases mixed with the H_2 were determined with Henderson's modification (1918) of Haldane's apparatus for analysis of expired air. The residue of gas not absorbed by KOH or pyrogallol was considered to be H_2. The pressure, p_{H_2}, of hydrogen, under which the cells or serum was saturated, was estimated as

$$p_{H_2} = \frac{\text{per cent } H_2 \text{ in tonometer gas}}{100} \times (B - 49.7)$$

where B represents barometric pressure in mm. of mercury, and 49.7 is the vapor tension of water at 38°.

The value of the Bunsen solubility coefficient, α, was calculated by the equation

$$\alpha = \frac{760}{p} \times \frac{[H_2]}{100} \times \frac{V_R}{V_{38°}} = \frac{756}{p} \times \frac{[H_2]}{100}$$

where p represents H_2 pressure at the time of saturation, calculated as above, $[H_2]$ is the volume per cent of hydrogen in solution, V_R and $V_{38°}$ represent respectively the volumes occupied by 1 gm. of the solution at room temperature and at 38°. The equation has been discussed in the accompanying paper on CO_2 solubility (Van Slyke, Sendroy, Hastings, and Neill (1928)). The H_2 determinations in the saturated fluids were all performed at a room temperature near 22° where the factor $\frac{V_R}{V_{38°}}$ is 0.995, and $\frac{V_R}{V_{38°}} \times 760 = 756$.

Our value of 0.01620 for α_{H_2} in water at 38° is 2.0 per cent below the value for that temperature interpolated from Winkler's data in the Landolt-Börnstein "Tabellen."

Fahr's (1911–12) value at 29.7° is 4 per cent lower than that interpolated from Winkler's data for that temperature. Our value for α_{H_2} in water accordingly lies, as nearly as one can estimate by interpolation, between Fahr's and Winkler's.

For ox serum at 38° our relative solubility, $\frac{\alpha \text{ observed}}{\alpha \text{ in } H_2O}$, of 0.946 is practically identical with Fahr's 0.950 at 29.7°.

In cells Fahr found relative H_2 solubility values at 29.7° from 0.86 to 0.90; ours at 38° are 0.891 and 0.904.

The α^0 values indicate cc. of H_2 dissolved per gram of water in solution. From these it is obvious that serum and cells, particularly the latter, dissolve more hydrogen than would be taken up by the water in them if the other constituents were inert. The values of the ratio $\frac{\alpha^0 \text{ observed}}{\alpha^0 \text{ in water}}$ show that serum dissolved 2.2 per cent more of the gas, and cells 18.8 per cent more, than the water present would by itself dissolve. The organic constituents

evidently dissolve considerable amounts. When we consider the depressant effect of the alkali salts on the solubility, the solvent power of the organic constituents stands out even more pronouncedly. In the last column of Table I the observed α^0 values

TABLE I.

Solubility of Hydrogen in Water, Serum, and Blood Cells at 38°.

Material.	H_2O content at 22°.	H_2 tension at 38°.	H_2 content (solution at 22° when analyzed).	α_{H_2} H_2 per cc. solution at 38°.	$\alpha^0_{H_2}$ H_2 per gm. H_2O in solution at 38°.	Relative solubilities.		
						$\frac{\alpha \text{ observed}}{\alpha H_2O}$.	$\frac{\alpha^0 \text{ observed}}{\alpha^0 H_2O}$.	$\frac{\alpha^0 \text{ observed}}{\alpha^0 \text{ saline}}$.
	gm. per cc.	*mm.*	*vol. per cent*	*cc.*	*cc.*			
H_2O	0.998	707	1.508	0.01613				
"	0.998	702	1.508	0.01624				
"	0.998	702	1.498	0.01613				
"	0.998	689	1.479	0.01623				
"	0.998	699	1.492	0.01614				
"	0.998	699	1.518	0.01642				
"	0.998	708	1.499	0.01601				
"	0.998	698	1.498	0.01622				
"	0.998	698	1.507	0.01632				
Average				0.01620	0.01632	1.000	1.000	1.036
0.15 M NaCl.	0.995	706	1.460	0.01563				
	0.995	706	1.452	0.01555				
Average				0.01559	0.01576	0.963	0.966	1.000
Ox serum.	0.924	694	1.403	0.01528	0.01662	0.943	1.018	
	0.924	694	1.413	0.01539	0.01676	0.950	1.026	
Average				0.01533	0.01669	0.946	1.022	1.058
Ox cells.	0.750	693	1.323	0.01443	0.01925	0.891	1.179	1.234
	0.750	693	1.342	0.01464	0.01952	0.904	1.196	1.252
Average				0.01454	0.01938	0.898	1.188	1.241

are compared with the α^0 values for the amount of physiological saline present. It there becomes apparent that the organic constituents increase the hydrogen taken up by serum by about 6 per cent, and that taken up by cells by about 24 per cent.

Stoddard (1926–27) has noted a similar behavior of the organic constituents toward nitrogen, and Van Slyke, Sendroy, Hastings, and Neill (1928) have observed it towards CO_2. The organic solvents for N_2 and CO_2 in the serum were identified as the lipoids, but the data of the above authors did not indicate in cells whether the lipoids or proteins or both were acting as gas solvents.

SUMMARY.

Ox serum was found to dissolve, per unit volume at 38°, 96.3 per cent as much hydrogen, and ox cells 89.8 per cent as much, as is dissolved by water. Per gram of water present, serum dissolved 6 per cent more, and cells 24 per cent more, than is dissolved per gram of water in 0.15 M NaCl solution. The organic constituents therefore dissolve considerable amounts of hydrogen.

BIBLIOGRAPHY.

Austin, J. H., Cullen, G. E., Hastings, A. B., McLean, F. C., Peters, J. P., and Van Slyke, D. D., *J. Biol. Chem.*, 1922, liv, 121.

Fahr, G., *J. Physiol.*, 1911–12, xliii, 417.

Henderson, Y., *J. Biol. Chem.*, 1918, xxxiii, 31.

Lundsgaard, C., and Schierbeck, K., *Am. J. Physiol.*, 1923, lxiv, 210.

Stoddard, J. L., *J. Biol. Chem.*, 1926–27, lxxi, 629.

Van Slyke, D. D., and Binger, C. A. L., *J. Exp. Med.*, 1923, xxxvii, 457.

Van Slyke, D. D., and Hiller, A., *J. Biol. Chem.*, 1928, lxxviii, 807.

Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523.

Van Slyke, D. D., Sendroy, J., Jr., Hastings, A. B., and Neill, J. M., *J. Biol. Chem.*, 1928, lxxviii, 765.

GASOMETRIC DETERMINATION OF HEMOGLOBIN BY THE CARBON MONOXIDE CAPACITY METHOD.

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The method here described is based on the capacity of hemoglobin to combine with the same maximum volume of CO as of O_2. The blood sample, which may vary from 0.1 to 2.0 cc., is saturated with CO in the 50 cc. chamber of the manometric blood gas apparatus of Van Slyke and Neill (1, 2). Approximately 2 cc. of carbon monoxide are introduced with the blood, and the chamber is evacuated and shaken. The carbon monoxide tension of approximately 25 mm. is sufficient to change the hemoglobin quantitatively to HbCO, while O_2 and N_2 are at the same time completely extracted from the blood. The gases are ejected, and the HbCO is determined by measurement of the CO set free by acid ferricyanide solution.

The corrections for physically dissolved O_2 and N_2 are eliminated by the above procedure. The substituted correction for physically dissolved CO is only 0.3 volume per cent, so that variations in it are negligible.

Economy of material and apparatus is attained by performing both the saturation with CO and its subsequent analysis in the chamber of the gas apparatus. The loss of part of the blood sample entailed in the usual oxygen capacity determination (1, 3, 4) by transfer after saturation in a separate vessel, is thus avoided. Consequently gasometric hemoglobin determinations are made possible with 0.1 or 0.2 cc. of blood.

Reagents.

Acid Ferricyanide Solution.—To 92 volumes of a stock solution containing 32 gm. of $K_3Fe(CN)_6$ per 100 cc. are added 8 volumes

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of concentrated lactic acid, of specific gravity 1.2. This concentrated acidified solution undergoes very slow decomposition, but can be used for over 2 months.[1]

Air-Free 1 N Sodium Hydroxide Solution.—A portion of this solution is made air-free (see p. 534 of Van Slyke and Neill (1)) each day and kept under oil, or is prepared less frequently and confined over mercury in the modified Hempel pipette shown in Fig. 1. For this purpose mercury replaces the water shown in Fig. 1, and the air-free solution replaces the CO gas.

5 N Sodium Hydroxide.—An approximately CO_2-free, saturated (18 N) NaOH solution is prepared by the usual procedure of dissolving NaOH in an equal weight of water, and permitting the solution to stand until the carbonate has settled. 27 cc. are diluted to 100 cc. to make the 5 N solution, which is kept protected from atmospheric CO_2 in the soda-lime tube, described previously (5).

Carbon Monoxide Gas.—This is prepared by warming a mixture of anhydrous formic and sulfuric acids. According to the reaction, $HCOOH = CO + H_2O$, each cc. of formic acid yields about 500 cc. of CO. In Fig. 2 is shown a simple arrangement for preparing and storing 3 liters of the gas, enough for 1500 analyses. The two 5 liter aspirator bottles are connected by a rubber tube, the bore of which should be as wide as 15 mm. in order to assure a flow of water sufficiently free to prevent the development of back pressure during generation of the CO. Before the latter process is begun the bottle, *A*, is filled completely with water. Sulfuric acid is dropped slowly into the formic acid in the tube, which is gently warmed with a micro burner. When about 300 cc. of mixed air and CO from the test-tube have collected in *A* the gases are ejected by opening the outlet cock of *A* and raising *B*. Then the rest of the sulfuric acid is run slowly into the formic acid, and the reaction is continued until the CO from all the formic acid has been

[1] In a portion of the acid ferricyanide solution, kept at room temperature in a flask of ordinary white glass and exposed to diffuse but not direct sunlight, the ferricyanide content was determined at intervals by measuring the N_2 gas evolved by the reaction with alkaline hydrazine solution. $4K_3Fe(CN)_6 + 4KOH + N_2H_4 = 4K_4Fe(CN)_6 + 4H_2O + N_2$. The percentages of original ferricyanide found were: 7 days, 98 per cent; 10 days, 93 per cent; 21 days, 92.3 per cent; 60 days, 91 per cent.

collected in *A*. The rubber tube connecting the test-tube with *A* is then closed with a screw clamp close to the glass inlet tube of *A*, and the test-tube is disconnected from *A*. *Because of the toxicity of CO the above operation should be carried out in a hood or where there is a free draft of air.*

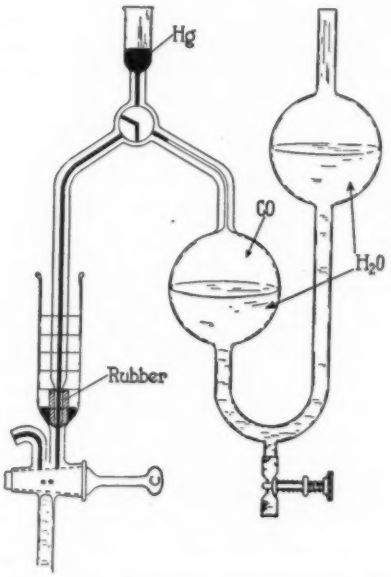

FIG. 1. Hempel pipette provided with 3-way stop-cock. The bulbs are of about 50 cc. capacity each. The capillary is of 1 mm. bore. When the pipette is not in use a little mercury is let into the capillary leading to the CO bulb, to seal the 3-way cock and prevent leakage around it. This drop of mercury in the capillary to the right of the cock is shown in the figure. This pipette may also be used to store air-free solutions. In this case the solution replaces the CO gas shown above, and mercury is used where water is indicated in the above figure. Supplied by the makers of the Van Slyke-Neill apparatus (1).

Procedure for Analysis of 2 Cc. Blood Samples.

Measurement of Blood Sample into Apparatus.—1 drop of caprylic alcohol is drawn into the capillary beneath the cup of the

manometric apparatus. (This capillary should be of only 1 mm. bore.) Into the cup are measured 4.75 cc. of water. With a stop-cock pipette, provided with a rubber tip (see Fig. 4, p. 532, of Van Slyke and Neill (1)), 2 cc. of blood are run directly into the chamber followed by the 4.75 cc. of water.

Measurement of CO into Chamber of Apparatus.—Approximately 2 cc. of CO, measured at atmospheric pressure, are required. This amount provides 0.50 cc. to combine with the maximum amount of hemoglobin ordinarily found in human

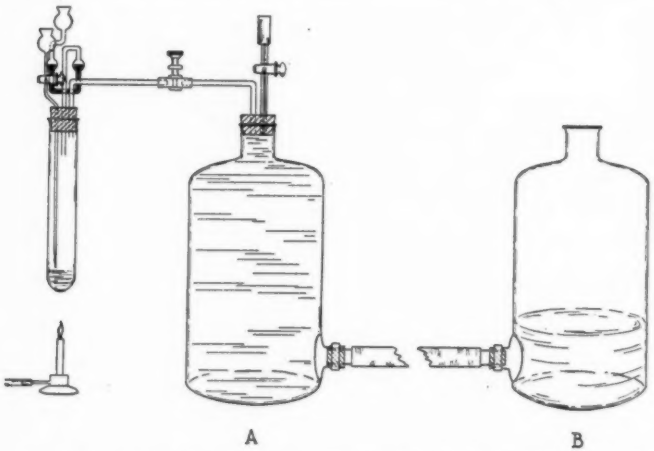

FIG. 2. Apparatus for the preparation and storage of carbon monoxide gas. The 100 cc. Pyrex test-tube where the gas is generated is provided with a mercury safety trap to prevent development of undue pressure.

blood, and leaves an excess of 1.5 cc., which is sufficient, when extended to 43 cc. of volume during the equilibration, to leave a CO pressure of about 25 mm. and insure quantitative change of Hb to HbCO. The exact amount of CO may vary from 2 cc. to 3 cc. if it is accurately duplicated in the blank analyses in which the correction for physically dissolved gases is determined. Any convenient device may be used for measuring the CO. The following has been found satisfactory.

From the Hempel pipette 2 cc. of the CO are measured into the chamber of the Van Slyke-Neill apparatus by the following pro-

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cedure. 1 or 2 cc. of mercury are placed in the cup above the chamber. The outlet capillary of the Hempel pipette is filled with mercury. The tip of the Hempel pipette is then fitted into the bottom of the cup, as shown in Fig. 1. The two cocks shown in Fig. 1 are now turned so that the CO gas can flow from the Hempel pipette into the chamber of the Van Slyke-Neill apparatus. The flow is regulated, not by either of the cocks shown, but by the cock leading to the mercury leveling bulb of the manometric apparatus. With the leveling bulb in the mid-position shown in Fig. 1 of Van Slyke and Neill's paper, this cock is opened slowly, and mercury is withdrawn from the chamber until CO gas has entered it as far as the 2 cc. mark. The gas is thus measured under a pressure indicated by the difference in height between the water columns in the two bulbs of the Hempel pipette. This difference may vary from about 10 cm. of water when the pipette is full of gas to 0 cm. when it is nearly empty. Such variation, however, amounts to only 0.01 of an atmosphere, which is negligible in its effect on the 0.30 volume per cent correction for dissolved CO.

Equilibration of Blood Solution with CO.—Both blood solution and CO gas having been measured into the chamber, the cock of the latter is closed and is sealed with a drop of mercury, and the chamber is evacuated. With the mercury meniscus at the 50 cc. mark, the chamber is shaken until equilibrium is reached. The time required varies somewhat with the speed of the motor. The necessary time is found by equilibrating for different periods in the blank analyses described below, and finding how long one must shake the chamber in order to reduce the blank to a minimum. In our apparatus this time is 1 minute. Equilibration being completed, the mixture of free gases, composed of all the O_2 and N_2, and part of the CO_2 from the blood, together with the excess CO, is ejected from the chamber.[2]

Determination of CO Bound as HbCO.—After ejection of the free gases, the chamber is evacuated until the blood solution is in the lower fourth, in order to keep the calibrated upper portion clean when the acid ferricyanide to be added precipitates methemoglobin. About 1 cc. of mercury and 2 or 3 cc. of water are placed

[2] For technique of ejection of gas see (6) foot-note 3, p. 240.

in the cup. Through the mercury seal 0.25 cc. of the acid ferricyanide solution is added from a micro burette graduated into 0.01 cc. divisions, and provided with a rubber-tipped delivery capillary.[3] Before the tip of the burette is inserted into the mercury it is moved through the supernatant layer of water, which dislodges any adherent ferricyanide crystals or air bubbles.

After addition of the ferricyanide, only enough mercury is admitted into the capillary beneath the cup to fill that capillary and the bore of the cock. It is preferable to have no mercury globules run down into the chamber at this time, because they are likely to adhere to the film of ferricyanide on the walls of the upper 2 cc. of space, which it is desirable to keep free and clean for subsequent gas measurement.

To extract the CO set free from HbCO by action of the ferricyanide, the mercury in the chamber is lowered to the 50 cc. mark, and the chamber is shaken slowly for about 5 seconds, to avoid too rapid foaming, then vigorously for 3 minutes.

Mercury is now admitted from the leveling bulb into the chamber until the gas space above the solution is reduced to 5 or 6 cc., and the CO_2 in the gas phase is absorbed with 1 cc. of air-free 1 N NaOH in the manner described on pp. 545–546 of Van Slyke and Neill (1).

The volume of the remaining gas, CO, with a slight trace of air introduced with the ferricyanide, is brought to 2 cc., and the pressure observed on the manometer is recorded as p_1. The gas is ejected.[2] The stop-cock of the chamber is again sealed with a drop of mercury, and the fluid meniscus is lowered to the 2 cc. mark. The manometer reading now observed, with the chamber gas-free, is recorded as p_2.

The hemoglobin content of the blood in terms of CO- or O_2-binding capacity is calculated by the equation

$$\text{CO or } O_2 \text{ capacity} = (p_1 - p_2 - c)f$$

f is a factor from Table II or III of Van Slyke and Neill (1) and c is a correction, determined by blank analysis, for the slight amounts of CO physically dissolved by the equilibrated blood solution, and of air admitted in solution in the 0.25 cc. of ferricyanide.

[3] Shown in Fig. 3 (2) p. 125 and described on p. 126.

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If the result is desired in terms of volumes per cent of O_2 or CO capacity, factor f is taken from the last column of Table II of Van Slyke and Neill (1). If the result is desired in terms of mm of O_2 or CO with which the hemoglobin of a liter of blood can combine, factor f is taken from the last column of their Table III.

The value of c is determined by a blank analysis in which the procedure described above is repeated in every detail, except that 2 cc. of water are substituted for the 2 cc. of blood. The correction c is calculated as

$$c = p_1 - p_2$$

The value of c is so small and insensitive to ordinary variations of laboratory conditions that, once exactly determined, it can be used as a constant. In our laboratory, with a temperature of 20–25° and barometric pressure of 740 to 780 mm., the value of c is 4.0 mm., of which 1.4 mm. are due to air admitted in the ferricyanide and 2.6 mm. to CO physically dissolved by the equilibrated blood solution (see Table III).

The cleaning of the chamber, after each analysis, is performed by rinsing with three successive portions of water, as described on p. 534 of Van Slyke and Neill's paper. To the first water portion a few drops of the 5 N NaOH are added to dissolve particles of methemoglobin adherent to the walls of the chamber. These particles usually form a narrow ring in the upper portion of the broad part of the chamber. They are dissolved in a few seconds by the alkaline solution, the chamber being shaken slowly, with enough mercury in it to raise the solution level up to the particles.

Procedure for Analysis of 1 Cc. Blood Samples.

The procedure is the same as when 2 cc. of blood are used, except that 2.5 cc. of water are taken instead of 4.75 cc., 0.13 cc. of the acid ferricyanide solution is used to liberate the CO, and 0.5 cc. of air-free 1 N sodium hydroxide is used to absorb the CO_2. The amount of CO added is the same, 2 cc. measured at atmospheric pressure. The c correction must be determined with these amounts of reagents. The calculation is the same, except that the factors in Tables II and III of Van Slyke and Neill (1) are found in the seventh column of each table, for the conditions that

the sample is 1 cc., S is 3.5 cc., and a is 2.0 cc., or the sixth column, when the CO pressure is determined with the gas at 0.5 cc. volume. With blood of less than ordinary hemoglobin content, more exact results will be obtained by using the 0.5 cc. a mark of the extraction chamber instead of the 2.0 cc. mark for the gas reading.

Procedure for Analysis of 0.1 or 0.2 Cc. Blood Samples.

The blood is drawn into a capillary pipette calibrated to *contain* either 0.1 or preferably 0.2 cc. (It should hold, when filled to the mark with dry mercury, 1.355 or 2.71 gm. of Hg.)

If the blood sample is drawn outside the laboratory, the pipette is delivered into a small test-tube, of about 1 or 2 cc. capacity, containing 0.5 cc. of water for a 0.1 cc. blood sample, 1.0 cc. of water for a 0.2 cc. sample. The pipette is rinsed twice by drawing up into it the supernatant water. The blood and water in the tube are then mixed with a fine rod or wire.

Before the sample thus diluted is transferred to the Van Slyke-Neill apparatus a drop of caprylic alcohol is drawn into the capillary below the cup of that apparatus. The blood solution from the test-tube is transferred to the cup, and thence, with the drop of caprylic alcohol, down into the chamber. The test-tube and cup are washed with repeated water portions of 4 or 5 drops each, until the volume of solution inside the chamber extends exactly to the 2 cc. mark.

If the blood is drawn in the laboratory, the pipette may be rinsed directly into 1 cc. of water previously placed in the cup of the gas apparatus. The blood is mixed with the water by stirring or shaking, and is delivered, together with a drop of caprylic alcohol, into the chamber. The cup is then washed with repeated small portions of water until the solution in the chamber reaches to the 2 cc. mark.

In the same manner described above for analysis of 2 cc. blood samples, 2 cc. of CO gas are added and equilibrated with the blood solution.

Of the ferricyanide solution only 0.05 cc. is added. It is run in while the blood solution is still in the top of the chamber, and is followed by several drops of mercury, which break up the methemoglobin precipitate into fine particles.

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The chamber is evacuated and shaken 3 minutes. The fluid is permitted to rise as near to the cock at the top of the chamber as it will with the leveling bulb at the mid-position. (See third paragraph, p. 546 of Van Slyke and Neill (1).) 3 or 4 drops of 5 N sodium hydroxide are run into the chamber, followed by a few drops of mercury. The alkali absorbs the CO_2 gas, and, mixed by the falling mercury drops with the dilute blood solution, dissolves the suspended methemoglobin particles. The aqueous meniscus is lowered below the 0.5 cc. mark, and then brought slowly up to it.

After the p_1 reading is taken, the gas is ejected,[2] the cock is sealed with mercury, the meniscus is again lowered to the 0.5 cc. mark, and p_2 is read on the manometer.

The correction, c, is determined by repeating the analysis without addition of blood.

For the *calculation* the factors in the fifth column of Table II or III of Van Slyke and Neill (1) are used, when 0.2 cc. of blood is employed. When the sample is only 0.1 cc. of blood, these factors are multiplied by 2.

EXPERIMENTAL.

Amount of Acid Ferricyanide Solution Required.—In order to minimize the correction for air introduced with the ferricyanide solution, and still avoid the necessity of preliminary extraction of its dissolved air, we have employed a minimum volume of a concentrated solution. The amount of ferricyanide required under the conditions of analysis was ascertained by preliminary experiments, in which 2 cc. portions of blood were treated as above described, except for variations in the amount of ferricyanide and lactic acid added. An example of the results is given in Table I. It is evident that a maximum yield of CO is obtained in Sample 3. As pointed out by Van Slyke and Neill (1), the equivalent of about 1 cc. of 0.1 N acid per cc. of blood must be added with the ferricyanide, to diminish the affinity of Hb for CO, or the CO will not be completely freed.

Tension of CO Required for Quantitative Saturation of Hemoglobin.

With 2 cc. samples of blood plus the usual 4.75 cc. of water in the chamber, varying tensions of CO gas were measured by the

following technique. The mercury in the chamber was lowered to the 50 cc. mark. Carbon monoxide from the Hempel pipette

TABLE I.

Amount of Ferricyanide Necessary to Liberate CO from 2 Cc. of Blood.

Oxygen capacity of blood by Van Slyke-Neill method = 23.27 volumes per cent.

Sample No.	Reagent used.				Volume of reagent added.	CO capacity found.
	Potassium ferricyanide.			Lactic acid, concentrated.		
	Amount used per analysis.	Concentration of solution used.	Volume.			
	mg.	*gm. per 100 cc.*	*parts by volume*	*parts by volume*	*cc.*	*vol. per cent*
1	38.4	8	96	4	0.50	22.83
2	46.0	20	92	8	0.25	22.92
3	76.75	32	92	8	0.25	23.29
4	153.5	32	96	4	0.50	23.15
5	153.5	32	92	8	0.50	23.20

TABLE II.

Tension of CO Required to Saturate Hemoglobin of Ox Blood, as Diluted for Analysis.

Blood No.	O_2 capacity by Van Slyke-Neill method.	Tension of CO at beginning of saturation.	Estimated volume of CO added, measured at 760 mm. and 22°.	Estimated tension of CO at end of saturation.	CO capacity found.	
	vol. per cent	*mm.*	*cc.*	*mm.*	*vol. per cent*	*per cent of O_2 capacity*
1	20.96	15	0.85	7	20.39	97.3
		30	1.70	22	20.97	100.0
		60	3.40	52	21.04	100.4
2	20.88	30	1.70	22	20.82	99.7
3	21.68	30	1.70	22	21.73	100.2
4	21.80	18.6	1.0	10	21.23	97.5
		35.3	2.0	27	21.73	99.8
		70.6	4.0	53	21.76	99.9

(Fig. 1) was then admitted until sufficient had entered to increase the pressure, read on the manometer, by the amounts indicated in Table II as "Tension of CO at beginning of saturation," the mer-

cury level in the chamber still remaining at the 50 cc. level. In fact, as the CO was admitted, the mercury in the chamber did not remain at this level, but fell as part of the mercury passed over into the rising column in the manometer. To bring the mercury back to the 50 cc. mark in the chamber, a little had to be admitted from the leveling bulb, with a consequent further rise also in the manometer column. We found that when sufficient gas was admitted to make the preliminary rise 70 per cent of the CO pressure desired, the above mentioned subsequent admission of mercury from the leveling bulb raised it to the desired pressure. The initial tension of CO could thus be regulated readily within 1 mm. The corresponding volumes of CO in cc. measured under atmospheric conditions are calculated in the fourth column of Table II, for comparison with the 2 cc. volume directed for routine use.

TABLE III.

Determinations of CO and Air in Blank Corrections of Analysis of 2 Cc. of Blood.

Pressures at 2.0 cc. volume.

Sample No.	$P_{CO + air}$ (a)	P_{air} (b)	P_{CO} (a) − (b)
	mm.	mm.	mm.
1	4.1	1.4	
2	3.9	1.4	
Mean.	4.0	1.4	2.6

The final CO tension was calculated by estimating the amount of CO left free in the chamber after the blood had absorbed the volume that was recovered in the subsequent analysis.

The results in Table II indicate that a final tension over 20 mm. is sufficient to insure complete saturation of the hemoglobin. At a final tension of 7 mm. 97.3 per cent was found saturated.

Relative Amounts of CO and Air in Blank Corrections.

Blank analyses were run as described for 2 cc. of blood. In each analysis 6.75 cc. of water were equilibrated in the gas chamber with 2 cc. of carbon monoxide gas. Then the free gases were ejected, 0.25 cc. of acid ferricyanide was added, the gases were extracted, and the pressure $P_{co + air}$ was measured at 2 cc. volume.

For P_{air} the same procedure was used, except that no CO was admitted. The gases measured by the pressure P_{air} after the second extraction in this case were derived from the air introduced with the 0.25 cc. of ferricyanide, plus a negligible trace of air not removed by the first extraction. The results are given in Table III.

Table IV indicates the order of constancy of the results obtained by the oxygen capacity method of Van Slyke and Neill (1) and by the present carbon monoxide capacity method applied with blood

TABLE IV.

Macro and Micro Determinations of CO Capacity of Blood Compared with O_2 Capacities by Van Slyke-Neill Method.

Blood No.	O_2 capacity.	CO capacity.			
	2 cc. samples.	2 cc. samples.	1 cc. samples.	0.2 cc. samples.	0.1 cc. samples.
	vol. per cent	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*
1 a	18.96	18.83	18.84	18.82	18.20
b	19.08	18.92	19.02	18.80	18.80
c	18.97	18.87	19.05	18.88	18.67
Average.	19.00	18.87	18.97	18.8	18.6
2 a	21.25	21.36	21.47	21.29	21.13
b	21.26	21.35	21.43	21.26	22.06
c	21.31	21.40	21.47	21.34	21.27
Average.	21.27	21.37	21.46	21.3	21.5
3a	22.35	22.52	22.50	22.29	22.70
b	22.45	22.42	22.38	22.51	22.31
c	22.37	22.42	22.29	22.50	22.20
Average.	22.39	22.45	22.39	22.4	22.4

samples of 2, 1, 0.2, and 0.1 cc. With blood samples down to and including 0.2 cc. the gasometric CO method, if carried out in detail according to directions, is probably more exact than any colorimetric method. When the sample is reduced to 0.1 cc., however, variations of as much as ± 2 per cent of the amount measured may occur in the gasometric procedure. In this case it has over colorimetric methods only the advantage of freedom from errors due to inaccurate or unstable colorimetric standards.

SUMMARY.

Hemoglobin is determined by measurement of its capacity to combine with carbon monoxide. The whole process of saturation of the blood with carbon monoxide and its subsequent analysis is carried out in the manometric gas apparatus of Van Slyke and Neill. Amounts of blood varying from 2 cc. to 0.1 cc. can be used.

The agreement of carbon monoxide capacities obtained by this method with oxygen capacities determined by the technique previously employed in this laboratory (1, 3, 4) confirms, with perhaps more exactness than previously available data, the fact that the hemoglobin of blood combines with identical volumes of oxygen and carbon monoxide.

BIBLIOGRAPHY.

1. Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, lxi, 523, (554).
2. Van Slyke, D. D., *J. Biol. Chem.*, 1927, lxxiii, 121.
3. Van Slyke, D. D., *J. Biol. Chem.*, 1918, xxxiii, 127.
4. Van Slyke, D. D., and Stadie, W. C., *J. Biol. Chem.*, 1921, xlix, 1.
5. Van Slyke, D. D., *J. Biol. Chem.*, 1927, lxxiii, 698.
6. Van Slyke, D. D., *J. Biol. Chem.*, 1926–27, lxxi, 235.

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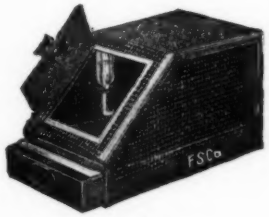

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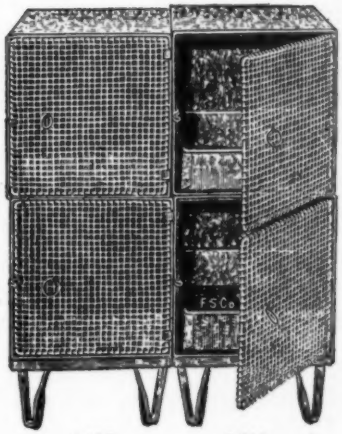

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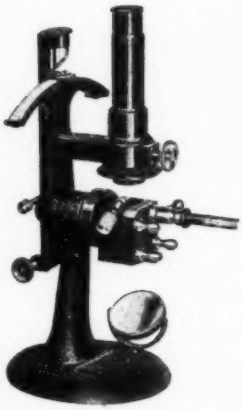

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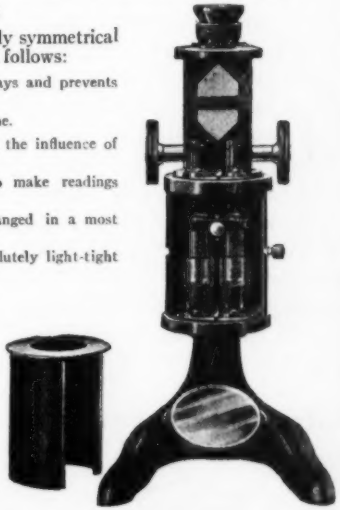

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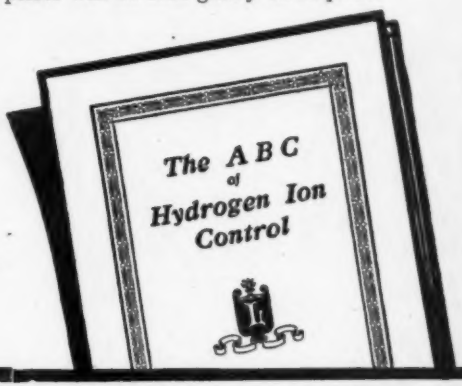

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